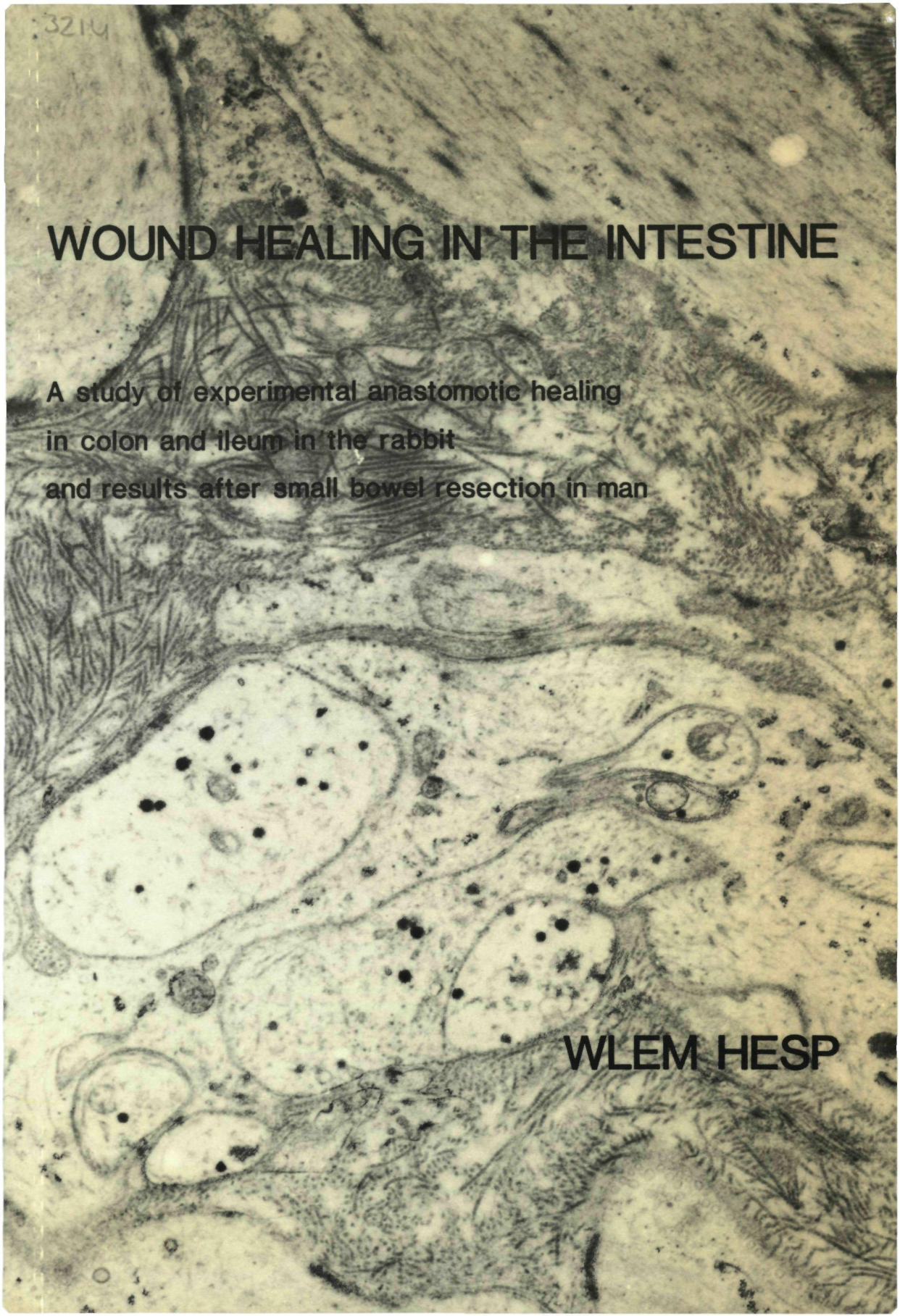

WOUND HEALING IN THE INTESTINE

A study of experimental anastomotic healing
in colon and ileum in the rabbit
and results after small bowel resection in man

WLEM HESP

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Walfridus Landoaldus Ericus Maria Hesp

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Cover: A 7 days old intestinal anastomosis.
(E.M. photograph. Magnification 20.400 x.
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CONTENTS

1. Healing of intestinal anastomoses: clinical data

Because of its high mortality and morbidity, anastomotic dehiscence is one of the most serious complications in gastrointestinal surgery. It causes one-third of the mortality after colorectal operations (1). The leakage rate for colonic anastomoses, as reported in some large series, varies between 4 and 14% (1,2,3,4,5). Systematic inspection by X-rays of all lower, extraperitoneal, anastomoses even reveals a leakage rate of 51%; fortunately, the large majority of these patients remains free of clinical symptoms (6). The seriousness of this complication is illustrated by the fact that 41% of the patients, who develop clinical signs of anastomotic leakage, eventually die and that the time of hospitalization doubles (5,7).

An abundance of literature exists concerning the results of colonic surgery. Proper technique (to work atraumatic), construction of well-vascularized wound edges, and prevention of tension are essential to the undisturbed healing of intestinal anastomoses. A number of factors are known which increase the risk of anastomotic dehiscence, e.g. the presence of infection (2,8,9), old age (2,3), a more distal localization of the anastomosis (2,3,7,10,11), improper bowel preparation (2,5,12), irradiation and residual tumor in the anastomotic area (2). Other factors, such as the type of anastomosis, the suture material, the use of antibiotics, the use of prednisone or the presence of diabetes mellitus appear to be of secondary importance. Procedures have been developed in order to avoid the problems concomitant to anastomotic construction in colonic surgery under high-risk conditions. In case of a difficult, not entirely reliable, anastomosis a diverting transverse colostomy will not prevent eventual leakage, but it will minimize its consequences with

regard to mortality and morbidity. Operating in stages is recommended, particularly in patients with acute complications of diverticulitis but also for those patients with an obstructing colonic carcinoma. After removing the diseased bowel, continuity of the intestine is not restored, but the proximal colon is brought out as an end colostomy. In case of an intraperitoneal resection the distal colon is attached distally in the wound as a mucous fistula (Mikulicz), in case of an extraperitoneal resection the stump is closed and buried under the pelvic peritoneum (Hartmann procedure). In a later phase, when conditions have improved sufficiently, continuity of the intestine may be restored.

In contrast with the ample documentation available in the literature concerning surgery of the large bowel, data on complications of small bowel anastomoses are almost completely non-existent. This should not simply be taken as indication that leakage of small bowel anastomoses seldom occurs. It seems also unlikely that such a leakage would be clinically unimportant. Kümmerle (13) reports a 100% mortality in 6 such patients and the mortality that goes with enterocutaneous fistula is hardly negligible (14). A possible explanation may be that in general practice surgery of the small bowel occurs far less frequently than surgery of the large bowel. Also, results of small bowel resections are difficult to find because in the literature they are given as secondary findings in papers primarily concerned with certain special diseases (e.g. Crohn's disease). Data on prevention and treatment of leakage of small bowel anastomoses are also hard to find. Reports are limited almost exclusively to pediatric surgery, particularly to patients with a neonatal necrotizing enteritis. Thus, some very basic questions remain to be answered concerning surgery of the small bowel. What is the incidence of anastomotic leakage? Do the risk factors which are known to affect adversely the healing of colonic anastomoses (e.g. infection) also challenge an undisturbed healing process

in the small intestine? Assuming that anastomotic leakage occurs less frequently in the small bowel than in the large bowel, what is the reason?

2. Healing of intestinal anastomoses: experimental studies

Anastomotic dehiscence is an example of disturbed wound healing. Our knowledge on the process of wound healing has become extensive (e.g. 15). However, most studies have been made on skin and fascial wounds. Their results are not necessarily valid for other tissues such as the gastrointestinal tract (16). Thus, experimental studies on gastrointestinal healing are needed to extend and complete our understanding of the repair process.

The macromolecule collagen plays a central role in the wound healing sequence (17). It also is the main component of the intestinal connective tissue layer, the submucosa, which provides the intestine with sufficient structural integrity and strength to withstand intraluminal pressures. Changes in collagen are thus very likely to affect the strength of the intestinal wall and a number of authors have reported on the effect of anastomotic construction on the collagen content of the large intestine. The picture that emerges is similar in most studies. During the first week after operation collagen levels, measured as hydroxyproline concentrations, decrease. Thereafter, they are gradually restored. Collagen loss is maximal 3 days post-operatively, at which time point the first measurement is usually taken. Relative maximal percentual decreases in collagen concentration reported are 19% (18) in rabbits, 52% (19) in dogs and 25 (20), 35 (21) and 43% (22) in rats. Loss of collagen is not restricted to the wound area, but may also be found in the colonic wall proximal to the anastomosis (21,22). Only Jiborn et al (23) have begun their measurements 2 days after operation. So far, no experimental data are available on the events which take

place during the first 48 hours after operation.

The results mentioned above indicate a high rate of collagenolysis after intestinal surgery. Both Cronin et al (24) and Irvin and Hunt (20) have investigated collagen breakdown by feeding rats ^{3}H-proline, part of which eventually ends up in collagen as ^{3}H-hydroxyproline. The changes in specific activity found in the anastomotic area 3 days after operation may be taken as evidence for the occurrence of collagenolysis, but do not allow any conclusions concerning the magnitude of this process. Hawley et al (18) have reported the presence of the enzyme collagenase, which is essential to the first step in collagen lysis, in the intestinal tract. They postulate an increased post-operative enzyme activity as a result of the disappearance of a circulating serum collagenase inhibitor (25).

Measuring the specific activity in hydroxyproline after feeding rats ^{3}H-proline 24 hours prior to sacrification, both Cronin et al (24) and Jiborn et al (23,26) have established significant increases in collagen synthesis as early as 2 days after anastomotic construction.

Taken together, these data indicate that the construction of a colonic anastomosis leads to a stimulation of both collagenolysis and collagen synthesis. Although the actual progress of both processes still needs to be quantitated, it seems clear that the net result is a very significant decrease in the amount of collagen around the anastomosis, which is at its maximum 3 days post-operatively. At first lysis dominates but approximately after 3 days synthesis starts to overtake lysis. Eventually, pre-operative collagen levels are restored after 7-14 days.

Concomitant to these events, the actual bursting strength of the anastomotic segment also appears to be greatly

diminished, as compared to that of the unwounded colon, during the first week after surgery (20,22,27). Furthermore, clinical experience learns that it is during this period that chances are greatest for anastomotic dehiscence to occur after intestinal surgery. Thus, the concept has been developed that anastomotic integrity is a race between collagen lysis and collagen synthesis (28). If lysis dominates dehiscence may result.

Retrospective studies have indicated a number of factors which may be detrimental to the undisturbed healing of intestinal anastomoses (2,3). Some of these factors have been further investigated in experimental models. The results of studies in animals with an induced infection appear equivocal. While Yamakawa et al (19) found increased leakage and collagen breakdown in dogs with experimental diverticulitis, Irvin (29) found no such effects in rats with an experimental peritonitis. Inflicting intra-abdominal trauma to rats also increased colonic leakage and decreases anastomotic collagen levels compared to those in untraumatized rats (30). However, there existed no significant difference between collagen concentrations of leaking and intact anastomoses. This emphasizes the fact that not only the collagen concentration is important for the strength of the intestinal wall, but also collagen structure and collagen type. It seems entirely plausible that the newly-formed collagen which must replace the mature collagen, lost during the first post-operative days, will need time to gain a stable structure.

The experimental studies mentioned before all pertain to colonic anastomoses. Data on experimental anastomoses of the small intestine are almost completely lacking. The only exception is a paper by Wise et al (31), which mentions the absence of a collagen loss from ileal anastomoses in dogs while colonic anastomoses indeed exhibit the lowered collagen concentrations also reported in other animals. Regrettably,

no factual data were given.

In conclusion, it appears that if one is interested in anastomotic healing in the small intestine, the first question to be answered is whether the collagenous equilibrium which is purported to be essential to colonic healing (28), also exists in experimental ileal anastomoses.

3. Aim of the study

The present study was initiated in order to examine the following points:

- Investigations into the healing of experimental colonic anastomoses start, with one exception, only 3 days after operation. In order to establish the entire course of healing, it seems necessary to investigate also the events taking place during the first 3 days, a period certainly important for the development of eventual subsequent anastomotic dehiscence.
- No data are available on the fate of collagen after anastomosis of the small intestine. Inventarisation of these events and comparison, also of histological features of wound healing, between ileal and colonic anastomoses may yield valuable indications as to cause and possible prevention of anastomotic leakage.
- Conflicting results exist concerning the effect of infection on healing of experimental intestinal anastomoses. Does induced infection indeed augment collagen lysis normally experienced after anastomotic construction?
- Clinical data on incidence of leakage of anastomoses of the small bowel are almost completely absent from the international literature. A retrospective study into incidence, prevention and treatment of leakage of small intestinal anastomoses certainly seems long overdue.

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Chapter 1

WOUND HEALING IN THE INTESTINAL WALL
A COMPARISON BETWEEN
EXPERIMENTAL ILEAL AND COLONIC ANASTOMOSES

W.L.E.M. Hesp, Th. Hendriks, E.J.C. Lubbers, H.H.M. de Boer
Dis Colon Rectum 27: 99-104, 1984

1.1 Summary

The healing of ileal and colonic anastomoses is compared in rabbits. The intestinal segment that contains the anastomosis shows a temporary loss of strength, which is reflected in a decreased bursting pressure. This loss of strength is accompanied by a massive loss of collagen, measured as hydroxyproline, both in ileum and in colon. In ileum, hydroxyproline concentrations, expressed on a dry weight basis, are lowered by 30 per cent, one day after operation. Thereafter, they rise again, after seven days reaching a level that is 40 per cent enhanced as compared with unwounded tissue. Maximal decrease in colon, measured two days after operation, is 40 per cent. After seven days hydroxyproline levels are back at preoperative values. In colon, significant loss of hydroxyproline is also apparent in the intestinal segment proximal to the anastomosis. This phenomenon does not occur in ileum. These results clearly demonstrate that after ileal anastomosis a loss of collagen occurs similar to that in colonic anastomoses. The fact that the loss of collagen is less extensive and more rapidly restored may be important in explaining the lesser incidence of leakage encountered after surgery of the small intestine.

1.2 Introduction

The fact that resection and anastomosis of the colon are

associated with a high incidence of anastomotic leakage is well documented (1-4). On the other hand, very few data are available concerning the leakage rate of ileal anastomoses. From this, one might infer that dehiscence of ileal anastomoses is not considered to be a frequent complication of surgery of the small bowel. The aim of current investigations in our laboratory is to define the molecular processes that cause the healing anastomosis to lose its strength in such a way as to result in leakage and, ultimately, to find ways of preventing these processes to occur.

The submucosa provides the intestine with sufficient structural integrity and strength to protect against intraluminal pressures (5). Since collagen is the predominant constituent of this connective tissue layer, it seems reasonable to assume that changes in collagen may affect the stability of the intestinal wall. Experimental studies have shown a massive loss of collagen from the anastomotic area during the first days after construction of a colonic anastomosis (6-9). This lysis of collagen is thought to be the underlying cause for the occurrence of anastomotic leakage.

It has already been mentioned that anastomotic leakage appears to be less frequent in ileum than in colon. This probably explains why there are so few data on the healing of ileal anastomoses. However, examination, description, and comparison of the phenomena occurring after construction of an anastomosis in both parts of the gut might contribute to our understanding of the processes responsible for disturbance of anastomotic integrity. The present report describes the changes in bursting pressure and hydroxyproline concentration that occur in the intestinal wall of rabbits after construction of an ileal anastomosis and compares these changes with those observed after construction of a colonic anastomosis.

For this study 72 male White New Zealand rabbits were used. The rabbits were divided into eight groups, four with ileal and four with colonic anastomoses, designated 1D, 2D, 3D and 7D, respectively, according to the time elapsed between operation and sacrifice. The number of animals in the various groups was four, four, 14 and 14, both in ileal and colonic groups 1D, 2D, 3D and 7D, respectively.

1.3.1 Operation

Surgical procedures were performed in sterile conditions. After an overnight fast, the rabbits were intubated and anesthetized with an oxygen-nitrous oxide-fluothane mixture. The abdomen was opened through a midline incision of approximately 4 cm.

Ileal anastomoses: The distal ileum was severed 10 cm proximal to the appendix tip. A 5 cm long segment (located proximally to the lesion) was removed for determination of bursting pressure and hydroxyproline content. Continuity was restored by an end-to-end anastomosis, using one layer of interrupted inverting sutures with Prolene^{R} 5-0. About 20 cm in a proximal direction, a second anastomosis was constructed similarly.

Colonic anastomosis: A 5 cm long segment was removed from the descending colon, for measurement of bursting pressure and hydroxyproline, and continuity was restored as described above. This anastomosis was placed 3 to 5 cm above the Douglas pouch. Subsequently, a second anastomosis was constructed 3 cm under the splenic flexure. Peritoneum and fascia were closed in one layer, with a continuous suture, using Vicryl 3-0, and the skin was closed with silk 2-0.

Postoperatively the animals were fed ad libitum. After one (n=4), two (n=4), three (n=14), or seven (n=14) days, rabbits in both (ileal and colonic) groups were killed by means of an overdose of pentothal. Then a 5 cm segment containing the first, more distal, anastomosis was removed for determination of bursting pressure and hydroxyproline content. In the rabbits with an ileal anastomosis a colonic segment was also collected (for hydroxyproline assay) from the position where, in the experimental group with colonic anastomosis, the lower anastomosis would be located. Likewise, an ileal segment was collected from the rabbits with a colonic anastomosis. The second, more proximal, anastomosis was used for examination of vascularization and histology (results not reported in this paper).

1.3.2 Bursting pressure

Fecal material was removed from the intestinal segment, which was then attached to an infusion pump and a device that recorded the internal pressure. Subsequently, pressure was increased by pumping an aqueous solution of methylene blue at a rate of 5 ml per minute into the segment, which was kept under water. Loss of pressure, indicating leakage, was recorded immediately and the site of perforation was indicated by the loss of methylene blue from the segment into the surrounding water. After determination of the bursting pressure, three 1 cm samples were collected from the intestinal segment for hydroxyproline assay: one containing the anastomosis and the other two representing the adjacent proximal and distal parts, respectively.

1.3.3 Hydroxyproline assay

All samples for hydroxyproline assay were frozen immediately in liquid N_2. Thereafter, samples were pulverized in a Braun microdismembrator, lyophilized and stored at $-30^{\circ}C$.

Hydroxyproline was measured essentially according to Prockop and Udenfriend (10). Between 2 and 5 mg lyophilized tissue was hydrolysed overnight at 120°C in 1.5 ml 6N HCl. The hydrolysate was filtered through 1.2 µm Sartorius membrane filter and two samples (volumes between 125 and 1000 µl) of the clear liquid were analyzed. Samples were brought to 1 ml with 6N HCl, phenolphtalein was added and the pH was raised to approximately 8.5 (light pink color) with KOH. One ml borate buffer, pH 8.7, and 0.5 ml 10 per cent alanine, pH 8.7, were added.

Samples were oxidized for 20 minutes at room temperature with 1 ml 0.2 M chloramine T in methyl cellosolve and 3 ml 3.6 M $Na_2S_2O_3 . 5H_2O$ was added to terminate the reaction. After extraction with 5 ml toluene the aqueous layer was heated for 25 minutes in a boiling water bath. Pyrrol was then extracted with 5 ml toluene and 1.2 ml Ehrlich's solution was added to 3 ml extract. Absorbance at 560 nm was measured after 20 minutes. Hydroxyproline content of samples was calculated from a linear standard curve, consisting of five standard hydroxyproline (Calbiochem, USA) samples (2 to 18 µg), which were processed similarly. Results were expressed on a dry weight basis. Sixty-two consecutive assays of a reference preparation of rabbit ileum resulted in an average hydroxyproline concentration of 10.5 µg/mg (range 9.0 to 12.3). The coefficient of variation was 6.8 per cent.

1.3.4 Materials

All materials used were of analytical grade.

1.4 Results

Average weight at operation was 2343 ± 255 (SD) g. Mean bursting pressure of the control segment, removed from ileum

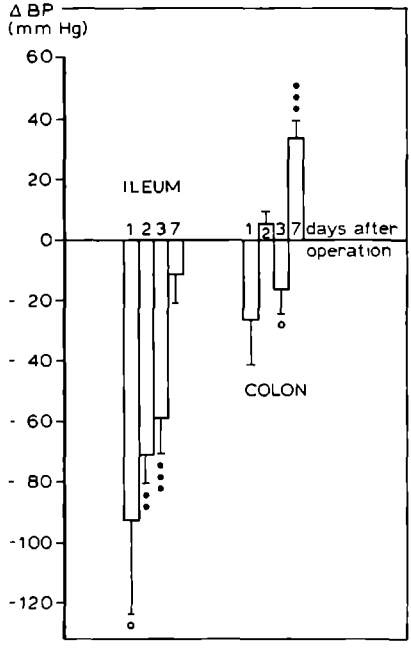

Figure 1:

Changes in intestinal bursting pressure after construction of an anastomosis. Bursting pressures (mmHg) of the intestinal segment around the anastomosis are measured at various times after operation and compared with preoperative bursting pressures. Results are expressed as average change (mmHg) with standard deviation for each group. Levels of significance are calculated by means of a paired sample t-test and represented in the following way:
o = $0.05 < p < 0.1$
●● = $0.001 < p < 0.01$
●●● = $p < 0.001$

during operation, was 180 ± 37 mm Hg, while the hydroxyproline content, calculated on a dry weight basis, averaged 8.9 ± 1.1 µg/mg. The average bursting pressure measured in unwounded colon was less than half (68 ± 34 mm Hg) of that found in ileum, while the hydroxyproline content was significantly higher (13.2 ± 1.8 µg/mg dry weight).

No significant differences in weight, bursting pressure, or hydroxyproline content were found within the control segments from either the ileum-operated or the colon-operated groups. Although the exact composition of intestinal collagen remains as yet unknown, a rough estimate of the collagen concentration may be derived from the hydroxyproline level by multiplying by a factor of 6.94 (11). This way, the collagen concentration approximated 62 µg/mg dry weight in ileum and 92 µg/mg dry weight in colon.

Absolute changes in intestinal bursting pressures are depicted

in Figure 1. Clearly, construction of an anastomosis leads to a loss of strength. In the ileum, bursting pressures after two and three days were significantly lower than those obtained peroperatively. After seven days the average bursting pressure was still slightly lowered. This loss of bursting strength was less proncunced after colonic anastomosis.

After three days a mean loss of 16 mm Hg occurred, which effect was almost significant (p=0.056, paired sample t-test). However, after seven days the bursting pressure was strongly elevated as compared with the colonic strength before operation. In order to compare more directly the size of the effect, in terms of percentual loss of strength, relative changes in small and large intestine are shown in Figure 2. Loss of strength of the intestinal wall seemed more pronounced in the ileum. Also, recovery of strength was slower in ileum than in colon. The temporary loss of strength of the anastomosis is also illustrated by the frequency of perforations occurring in the anastomosis as compared with perforations occurring outside the proper healing area (Table 1). While three days postoperatively the intestine frequently perforated in the anastomosis, seven days after operation the strength of the anastomotic area was always greater than that of the adjacent wall.

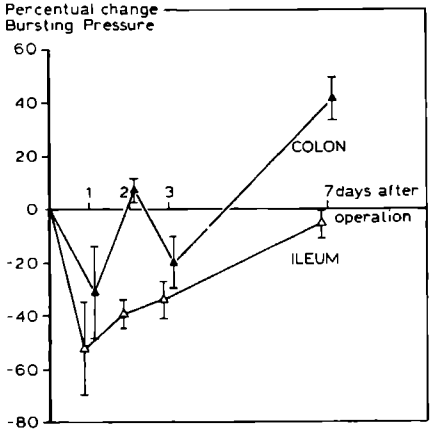

Figure 2:

A comparison of relative changes in bursting pressure caused by ileal or colonic anastomosis. The mean percentual change in bursting pressure of the anastomotic segment, calculated with respect to the pre-operative value, is depicted together with the standard deviation after construction of an ileal (Δ) or colonic (▲) anastomosis.

Table 1: *Bursting site in intestinal segments containing an anastomosis.*

	Group*	Bursting within anastomosis	Bursting elsewhere	p
Ileum	3D	10	4	0.0002
	7D	0	14	
Colon	3D	7	7	0.0058
	7D	0	14	

* A comparison is made between the 3D and 7D groups regarding the frequency of perforations occurring within the anastomotic area. Level of significance is calculated with Fisher's exact test for the two times two table.

We examined the hydroxyproline content, not only of the anastomotic area, but also of both (1 cm) proximal and distal segments. The absolute changes in hydroxyproline content, expressed in µg/mg dry weight, for the groups with ileal anastomoses are given in Figure 3. Changes were most pronounced in the anastomotic area. Already, after one day, loss of hydroxyproline was maximal. While after three days decrease of hydroxyproline was limited, levels were still significantly lower than before operation. Apart from an almost significant ($p=0.06$, paired sample t-test) effect in the proximal segment after one day, no significant loss of hydroxyproline was measured in proximal or distal segments.

Seven days after operation the collagen content in all segments was strongly enhanced as compared with levels in unwounded tissue. Figure 4 shows similar data for the experimental groups with a colonic anastomosis. Again, hydroxyproline levels in the anastomotic segment were most strongly affected. Maximum loss of hydroxyproline was found after two days. Significant loss of hydroxyproline also

Figure 3:

Changes in hydroxyproline content of intestinal segments after construction of an ileal anastomosis. Hydroxyproline is measured immediately around the anastomosis (A) and in a proximal (P) and a distal (D) segment and compared with the preoperative values. Results are expressed as average change (µg/mg dry weight) with standard deviation. Levels of significance for the differences found are calculated by means of a paired sample t-test and represented in the following way:

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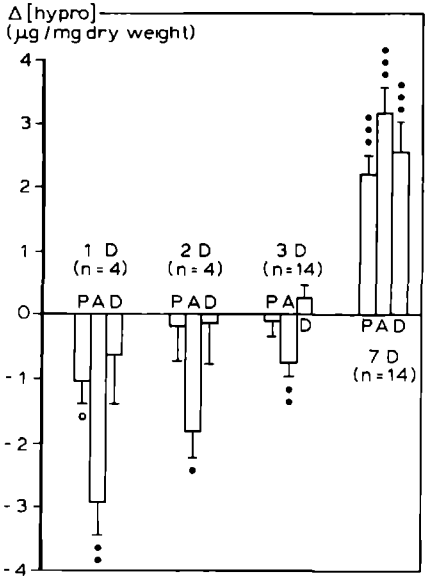

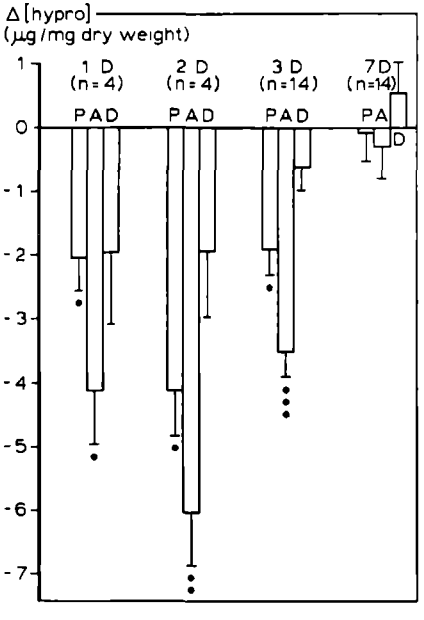

Figure 4:

Changes in hydroxyproline content of intestinal segments after construction of a colonic anastomosis. Hydroxyproline is measured immediately around the anastomosis (A) and in a proximal (P) and a distal (D) segment and compared with the preoperative value. Results are expressed as average change (µg/mg dry weight) with standard deviation. Levels of significance for the differences found are calculated by means of a paired sample t-test and represented in the following way:

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●● = 0.001 < p < 0.01
●●● = p < 0.001

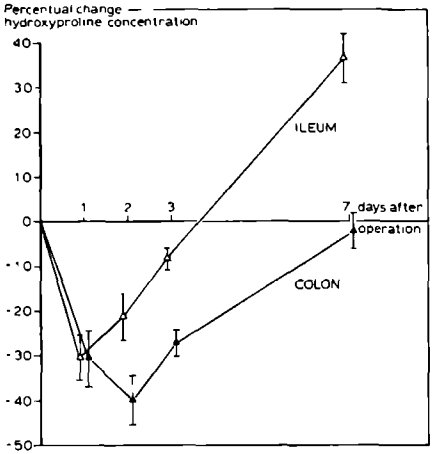

Figure 5:

A comparison of relative changes in hydroxyproline concentration around ileal and colonic anastomoses. Depicted is the mean percentual change in hydroxyproline level of the anastomotic segment, calculated with respect to the preoperative value, together with the standard deviation after construction of an ileal (Δ) or colonic (▲) anastomosis.

occurred in the proximal segments. While mean values in distal segments were also lowered, standard deviations were too large to pronounce these effects significant. After seven days hydroxyproline levels were back to normal.

Relative changes in hydroxyproline content of the anastomotic area in ileum and colon are compared in Figure 5. In direct contrast to the decrease in bursting strength, which was greater and was undone more slowly in ileal segments, the loss of hydroxyproline was more massive and less quickly compensated for in colonic segments. We have also compared values of bursting pressure and hydroxyproline content in all experimental groups, but found no significant correlation, either positive or negative, between the two.

Finally, it was investigated if surgery of one part of the intestine would affect hydroxyproline levels in another distant part. Table 2 shows that operation in either ileum or colon did not change hydroxyproline concentrations in colon or ileum, respectively.

Table 2: *Effects of construction of ileal or colonic anastomosis on hydroxyproline levels in distant intestinal segments.*

Group		Number of rabbits	Hydroxyproline* (µg/mg dry weight)
Unwounded ileum		36	8.9 ± 1.1
Ileal segment after colonic anastomosis	1D	4	8.3 ± 1.4
	2D	4	9.5 ± 1.0
	3D	14	9.2 ± 1.1
	7D	12	9.3 ± 1.0
Unwounded colon		36	13.2 ± 1.8
Colonic segment after ileal anastomosis	1D	4	13.7 ± 0.4
	2D	4	13.5 ± 1.3
	3D	12	12.6 ± 2.2
	7D	14	14.1 ± 1.2

* Hydroxyproline concentrations in ileum after colonic anastomosis are compared with levels in unwounded tissue, resected during construction of ileal anastomoses. Data for hydroxyproline levels in colon are obtained in a similar way. Results are expressed as mean values with standard deviation. No statistically significant differences exist, either if unwounded ileum is compared with ileal segments after colonic anastomosis or if unwounded colon is compared with colonic segments after ileal anastomosis (Wilcoxon two-sample test).

1.5 Discussion

A most important factor in healing of intestinal, particularly colonic, anastomoses is thought to be the presence of adequate amounts of collagen. In fact healing is described as a struggle between collagen lysis and collagen synthesis (12). Impaired healing or leakage of anastomosis would then primarily be

caused by a defect in collagen metabolism. The first phase of healing of colonic anastomosis appears to be characterized by a significant loss of collagen (6-9), which protein has been reported to possess a very low turnover rate in intact intestine (13).

Hawley et al (7) reported a 19 per cent loss of hydroxyproline in colonic anastomoses in rabbits three days after operation. We have found a loss of 28 per cent at this stage of wound healing (Figure 5). Most experiments on collagen breakdown after anastomotic construction describe hydroxyproline measurements after three days. Only Jiborn et al (14), working with rats, examined hydroxyproline concentrations two days postoperatively and found a decrease similar to that observed after four days. Our results show that already, one day after operation, a significant loss of approximately 30 per cent of colonic hydroxyproline has occurred. After two days the hydroxyproline concentration is at its lowest level, and at three days synthesis seems to have overtaken breakdown. Thus, an immediate and massive loss of collagen after colonic injury appears well documented. A recent claim by Stromberg and Klein (15) that collagen mass does not appear to be decreased because of surgical injury seems unjustified, more so since the authors base their conclusion on measurements performed three weeks after anastomotic construction.

Experiments on changes in collagen in ileal anastomoses have not been reported before. Although Wise et al (16) state that they found no significant decrease in the hydroxyproline content of small intestinal anastomoses in dogs, they fail to supply any experimental data, thus preventing meaningful evaluation of their results. Our experiments indicate that in ileal anastomoses a pattern of hydroxyproline loss exists similar to that in colonic anastomoses. In ileum, a significant decrease in hydroxyproline concentration in the anastomotic segments is apparent after one, two and three

days (Figure 3). The loss of hydroxyproline is restricted almost completely to the actual area of wounding. While in colon the segment proximal to the anastomosis also shows a significant decrease in hydroxyproline during the first three days (Figure 4), this effect is only indicated on day 1 in ileum (Figure 3). Thus, essentially the same phenomenon occurs after both ileal and colonic anastomosis. Ileal loss of collagen appears quantitatively smaller and less extensive and recovery seems to proceed more rapidly.

Hawley et al (7) have suggested that increased collagenase activity following injury is not a local process. They reported significantly elevated levels of collagenase in the small intestine after colonic anastomosis in rabbits. However, they did not report actual measurements of hydroxyproline concentrations in distal intestinal segments. We have found no evidence supporting the thesis that local injury produces collagen breakdown in the intestinal wall at locations other than those close to the healing area (Table 2). Ileal anastomosis did not result in any change in colonic hydroxyproline; neither did colonic anastomosis after ileal hydroxyproline. While it seems possible that the in vitro activity of collagenase is enhanced, it appears that in vivo this does not lead to a loss of collagen.

The mechanical strength of the intestinal wall may be evaluated by measuring the bursting pressure, which is a measure of the wall resistance to increasing intraluminal pressure. It has been suggested (17) that the bursting wall tension, which means the circular wall tension at the point of rupture, gives a more accurate measurement of bursting strength. However, comparison of both parameters in studies of changes of bursting strength after colonic anastomosis have shown a good correlation between the two (8,18). Therefore, we have examined the bursting pressure, which is easier to measure. Loss of intestinal strength after anastomosis is apparent, both in

ileum and in colon. Percentual changes in bursting pressure are greater in ileum than in colon (Figure 2). Also, colonic segments appear to regain strength more quickly. The fact that this temporary loss of strength is located in the anastomosis is illustrated by the finding that three days postoperatively most of the ruptures occur in this area, while seven days postoperatively all segments rupture elsewhere (Table 1), indicating that at this point the anastomosis has grown stronger than the adjacent bowel wall.

Thus, after anastomotic construction, a transient loss of strength is accompanied by a temporary loss of collagen, both in ileum and in colon. Comparison of Figures 2 and 5 shows that changes in bursting pressure are more pronounced in ileum, while loss of hydroxyproline seems more extensive in colon. The absence of a direct correlation between bursting pressure and hydroxyproline (collagen) level does not necessarily mean that loss of intestinal strength is not caused by changes in the collagen matrix. It merely indicates that not the absolute concentration, but rather structure (cross-linking) and type of collagen present may be the decisive factors in establishing integrity of tissue. Currently, experiments are in progress in our laboratory in order to evaluate the changes in these parameters induced by injury of the intestinal wall.

Adopting the hypothesis that anastomotic integrity is determined by the ratio of collagen breakdown to collagen synthesis (12), it is tempting to correlate the lesser incidence of dehiscence of ileal anastomoses with the less massive changes in hydroxyproline levels as compared with those observed in colon. However, more data should be collected in order to substantiate this, in our opinion, attractive proposal.

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LOSS OF COLLAGEN FROM EXPERIMENTAL INTESTINAL ANASTOMOSES: EARLY EVENTS

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Exp Mol Pathol 42: 411-418, 1985

2.1 Summary

Collagen lysis, which always occurs to some extent in the wound area, is thought to be the underlying cause for breakdown of intestinal anastomoses. Therefore, we have studied the loss of collagen around ileal and colonic anastomoses in New Zealand White rabbits during the first 48 h after operation.

In ileum, significant lysis of collagen in the anastomotic area as represented by a decreased level of hydroxyproline, occurs from 12 h post-operatively onwards. Maximal loss of hydroxyproline, as compared to pre-operative values, is 27% measured 24 h after operation.
In colon, significant lysis of collagen occurs already after 3 h. The lowest level of hydroxyproline measured during the experimental period is found 48 h after operation, where the concentration is decreased by 38%. Changes in ileum are restricted to the anastomotic area, while in colon the decrease in hydroxyproline extends along the intestinal wall, particularly in proximal direction. The fact that total protein concentrations do not vary significantly indicates the lowered hydroxyproline levels to be specific.

Microscopic examination of the wound area shows that the cellular response during the first 24 h after wounding is restricted to granulocytes. It is suggested that granulocyte

collagenase is mainly responsible for the observed lysis of collagen after intestinal anastomosis.

2.2 Introduction

Anastomotic leakage in the intestine seems to occur almost exclusively in the colon. While the high incidence of leakage of colonic anastomoses is amply documented (1), we have found no data in the literature concerning the leakage rate of ileal anastomoses. From this, one might infer that dehiscence of ileal anastomoses is not considered to be a frequent complication of surgery of the small bowel. Therefore, inventarisation and comparison of the phenomena occurring after construction of an anastomosis in both parts of the gut might yield valuable information concerning the processes responsible for disturbance of anastomotic integrity.

The connective tissue macromolecule collagen plays a central role in wound healing (2). The underlying cause for the occurrence of anastomotic leakage is thought to be a change in collagen metabolism, resulting in a massive loss of collagen from the anastomotic area during the first days after construction of the anastomosis. Earlier work has shown that such a process occurs around experimental colonic anastomoses (3), the first measurements being performed two days after operation (4). We have shown recently that after ileal anastomosis a similar loss of collagen occurs, despite the aforementioned fact that clinical signs of leakage of small bowel anastomoses apparently are not or hardly ever seen (5).

Accepting the hypothesis that lysis of collagen around the anastomosis may ultimately lead to such a weakening of the integrity of the intestinal wall that dehiscence may result, it seems logical to investigate whether it is possible to decrease the anastomotic leakage rate by diminishing collagen

breakdown. As a first step, characterization of the collagenolysis and identification of factors which influence this process appear necessary. The present report documents the occurrence of substantial collagenolysis, reflected by decreased concentrations of hydroxyproline, within the first 24 h after construction of both ileal and colonic anastomoses in the rabbit.

2.3 Materials and methods

For this study, 73 male New Zealand White rabbits were used with an average weight of 2380 g (SD: 244, range: 1800-3000 g); 41 rabbits received an ileal anastomosis, 32 a colonic anastomosis.

Surgical procedures were performed in sterile conditions. After an overnight fast the rabbits were intubated and anesthesized with an oxygen-nitrous oxide-fluothane mixture. The abdomen was opened through a mid-line incision of approximately 4 cm.

Ileal anastomosis: the distal ileum was severed 10 cm proximal to the appendix tip. A 2 cm long segment (located proximally to the lesion) was removed for determination of hydroxyproline content. Continuity was restored by an end-to-end anastomosis, using one layer of interrupted inverting sutures with Prolene^{R} 5 x 0. About 20 cm in proximal direction a second anastomosis was constructed similarly.

Colonic anastomosis: a 2 cm long segment was removed from the descending colon, for measurement of hydroxyproline, and continuity was restored as described above. This anastomosis was placed 3-5 cm above the Douglas pouch. Subsequently a second anastomosis was constructed 3 cm under the splenic flexure. Peritoneum and fascia were closed in one layer, with a continuous suture, using Vicryl 3 x 0 and the skin was closed

with silk 2 x 0.

Postoperatively the animals were fed ad libitum. The animals with an ileal anastomosis were sacrificed, by means of an overdose of penthotal, after 3 h (n=5), 6 h (n=5), 12 h (n=6), 15 h (n=6), 18 h (n=6), 24 h (n=7) and 48 h (n=6). Rabbits with a colonic anastomosis were sacrificed after 3 h (n=6), 6 h (n=7), 12 h (n=6), 24 h (n=7) and 48 h (n=6). The intestinal segment containing the first, more distal, anastomosis was removed and three 1 cm samples were collected for hydroxyproline assay: one containing the anastomosis and the two representing the adjacent proximal and distal parts, respectively. The second, more proximal, anastomosis was used for histology.

All samples for biochemical assays were frozen immediately in liquid nitrogen. Subsequently, samples were pulverized in a Braun microdismembrator, lyophilized and stored at $-30^{o}C$.

Hydroxyproline was measured (in duplicate) essentially according to Prockop and Udenfriend (6), using a hydroxyproline standard from Calbiochem (USA). Protein was assayed using bovine serum albumine (Type V, Sigma, USA) as a standard (7).

Anastomotic segments for histology were removed and collected in 4% (w/v) formaldehyde. Samples were dehydrated with, successively, acetone, methyl benzoate and toluene and fixed in paraffin. Thereafter, they were sliced into 6 µ sections, which were stained with hematoxylin-eosine and screened for occurrence of various cell types.

Methods used for statistical evaluation of the data are given together with results in the appropriate section.

The average hydroxyproline concentration in unwounded intestine is 8.01 (SD: 1.42, range: 5.69-12.25, n=41) µg/mg dry weight for ileum and 13.04 (SD: 2.14, range: 9.48-16.58, n=32) µg/mg dry weight for colon.

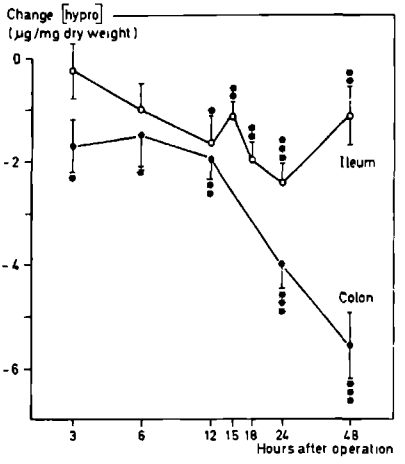


Figure 1:

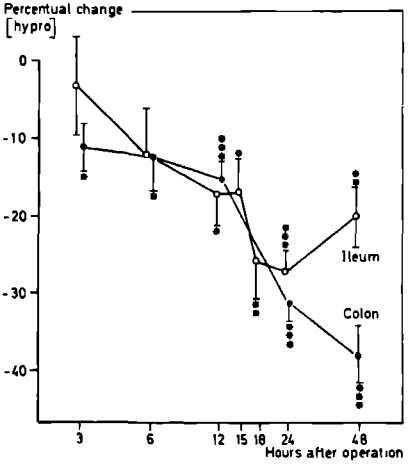
Absolute changes in hydroxyproline content of anastomotic segments during the first 48 h after operation. Results are expressed as average change (µg/mg dry weight) with standard error of the mean; the time scale is logarithmic. Levels of significance for the differences between anastomosis and unwounded tissue are calculated by means of a paired-sample two-sided Student t-test and represented in the following way:

* $0.01 < p \leq 0.05$
** $0.001 < p \leq 0.01$
*** $p \leq 0.001$

Figure 2:

Relative changes in hydroxyproline content of anastomotic segments during the first 48 h after operation. Results are expressed as mean percentual change, calculated with respect to the pre-operative value, with standard error of the mean; the time scale is logarithmic. Levels of significance for the differences between anastomosis and unwounded tissue are calculated by means of a paired-sample two-sided Student t-test and represented in the following way:

* $0.01 < p \leq 0.05$
** $0.001 < p \leq 0.01$
*** $p \leq 0.001$



The variations in hydroxyproline levels measured in the anastomotic area during the first 48 h after construction of an anastomosis are represented both as absolute, Figure 1, and relative, Figure 2, changes. Statistical comparison by means of a paired-sample two-sided Student t-test, of the values found at operation and at sacrifice shows similar results with both approaches. In the ileal segment, hydroxyproline levels in the anastomotic area are significantly lowered from 12 h after operation. In colon, this decrease is significant already 3 h after operation.

The course of the hydroxyproline loss in both intestinal segments was analyzed further by means of one-way analysis of variance. In ileum, it appears that the groups of measurements obtained at the 7 time points, which are represented by their mean values in Figures 1 and 2, cannot be considered to be random samples from the same population ($p=0.0164$), which means that hydroxyproline levels change significantly over this time period. Further comparison of the groups at the various time points by means of the contrast test of Tukey (8) shows the loss of hydroxyproline at 18 and 24 h after operation to be significantly greater than the loss 3 h post-operatively. Maximal decrease of hydroxyproline levels occurs 24 h after operation and amounts to 27% of the pre-operative value. The same statistical procedures have been applied to the 5 groups of data, obtained for colonic anastomosis at various times after operation. Analysis of variance shows a very significant ($p<0.001$) change in hydroxyproline levels with time during the period of investigation. Loss of hydroxyproline at 24 and 48 h is significantly greater than that 3, 6 or 12 h after operation. Here maximal lowering of hydroxyproline, amounting to 38% of pre-operative values, was found 48 h post-operatively.

It is also possible to compare the course of hydroxyproline changes around ileal and colonic anastomoses by means of

Table 1: *Postoperative changes in hydroxyproline levels in segments proximal and distal to intestinal anastomoses. Only those experimental groups are represented which show a significant or almost significant effect in either one or both segments, as calculated by means of a paired-sample two-sided Student t-test.*

	n	segment	concentration change (µg/mg dry weight ± SEM)	level of significance
ILEUM				
24 h	6	proximal	-0.84 ± 0.36	p = 0.06
COLON				
3 h	6	proximal	-1.43 ± 0.62	p = 0.07
6 h	7	proximal	-1.16 ± 0.50	p = 0.06
12 h	6	distal	-0.85 ± 0.41	p = 0.09
24 h	7	proximal	-2.49 ± 0.39	p < 0.001
		distal	-1.68 ± 0.67	p = 0.04
48 h	6	proximal	-3.61 ± 0.73	p = 0.004
		distal	-2.16 ± 0.72	p = 0.03

two-way analysis of variance, which was performed using the data obtained in both ileum and colon 3, 6, 12, 24 and 48 h after operation. The loss of hydroxyproline appears to be systematically greater around colon anastomoses, both if absolute changes ($p<0.001$) and relative changes ($p=0.03$) are used for the comparison. Also, the shape of the time curve for the ileal anastomoses is significantly ($p=0.005$) different from the shape of the curve for colonic anastomoses, if absolute changes are compared (cf. Figure 1).

In ileum, changes in hydroxyproline levels are almost completely restricted to the anastomotic area. The only possible exception is the proximal segment 24 h after operation, where the average loss of hydroxyproline is almost ($p=0.06$) significant (Table 1). In colon, the decrease in hydroxyproline

Table 2: *A comparison of protein concentration in unwounded tissue and anastomotic segments in the various experimental groups. Data are represented as mean value with standard error of the mean. Statistical evaluation of the difference between unwounded and anastomotic tissue is performed with a paired-sample two-sided Student t-test.*

		protein concentration (µg/mg dry weight)			
	n	unwounded tissue	anastomosis	difference	level of significance
ILEUM					
3 h	5	710 ± 30	744 ± 36	34 ± 14	p = 0.07
6 h	5	603 ± 61	681 ± 80	78 ± 59	n.s.*
12 h	6	686 ± 27	677 ± 17	-9 ± 36	n.s.
15 h	6	730 ± 33	733 ± 25	3 ± 50	n.s.
18 h	6	679 ± 46	673 ± 69	-6 ± 31	n.s.
24 h	7	676 ± 48	680 ± 50	4 ± 30	n.s.
48 h	6	700 ± 16	731 ± 22	31 ± 26	n.s.
COLON					
3 h	6	772 ± 49	826 ± 39	54 ± 37	n.s.
6 h	7	685 ± 28	712 ± 33	27 ± 13	p = 0.08
12 h	6	706 ± 36	715 ± 33	9 ± 33	n.s.
24 h	7	692 ± 30	662 ± 47	-30 ± 60	n.s.
48 h	6	665 ± 13	630 ± 33	-35 ± 40	n.s.

*n.s. = non significant, 0.1 < p

measured in the anastomotic area extends further along the intestinal wall, particularly in proximal direction. These effects are most pronounced from 24 h post-operatively onwards (Table 1).

We have also measured total protein concentrations in control segments, obtained at operation, and anastomotic segments. The protein concentration averages 685 (SD: 96, range: 498-842, n=41) µg/mg dry weight in unwounded ileum and 703 (SD: 86, range: 561-966, n=32) µg/mg dry weight in colon. From this,

Table 3: *Occurrence of different types of cells in sections of intestinal anastomoses at various times after operation.*

	n	granulocytes	monocytes/ macrophages	fibroblasts
ILEUM				
3 h	5	+	-	-
6 h	5	+	-	-
12 h	6	++	-	-
15 h	3	++	-	-
18 h	4	++	-	-
24 h	3	++	±	+
48 h	3	+	+	++
COLON				
3 h	4	+	-	-
6 h	7	+	-	-
12 h	2	++	-	-
24 h	3	+	±	+
48 h	3	+	+	++

- absent
± occasional cell present
\+ present
++ abundantly present

it can be calculated that collagen comprimes approximately 8% and 13% of total protein in the wall of ileum and colon, respectively. Table 2 shows that no significant changes in total protein occur, with the possible exception of a slight increase in ileum 3 h and in colon 6 h after operation.

The occurrence of various types of cells in the wound area has been examined microscopically in the second set of, more proximally constructed, anastomoses. The results are summarized in Table 3. A similar picture arises for both ileum and colon. Granulocytes are present immediately after wounding. Their

number increases up until 12-24 h post-operatively. An occasional monocyte may be observed after 24 h, but their number starts to grow significantly only between 24 and 48 h. The same goes for fibroblasts, although a number of these cells is present already 24 h after operation.

2.5 Discussion

A lowered hydroxyproline concentration around experimental colonic anastomoses, as measured from 2 days post-operatively onwards, has been documented before (4). Stromberg and Klein (9) have argued that a change in hydroxyproline concentration does not necessarily mean that the actual amount of hydroxyproline changes: a decreased concentration might also be caused by an increase in wet weight or dry weight. In our study total protein concentrations do not change significantly in any of the experimental groups (Table 2). This supports the conclusion that the hydroxyproline effect is rather specific and indicative for true lysis of collagen.

The present data represent the first description of changes in collagen levels during the initial 48 h after construction of intestinal anastomoses. Figure 1 shows that the lysis of collagen in the anastomotic area starts immediately after wounding, at least in colon. So far, changes in collagen concentration during wound healing after anastomosis of the small bowel have not been described in the literature. Our results clearly indicate that a similar process occurs in the ileum, although it takes 12 h before hydroxyproline levels have decreased significantly. The absolute size of the effect appears greater in colon than in ileum. Since the concentration of hydroxyproline in ileum is lower than in colon, the relative size of the effect, at least from 6 to 24 h after operation, appears about equal in both intestinal segments (Figure 2). Still, statistical comparison of the data obtained over the entire experimental period show the loss of hydroxy-

proline to be systematically greater around colonic anastomoses. Apart from the apparent slower onset of the collagenolysis, the duration of a state of decreased hydroxyproline levels seems shorter in ileum. After 24 h the concentration starts to rise again, while in colon the lowest point is at 48 h after operation. Moreover, pre-operative levels are restored after 3 days in ileum and only after 7 days in colon (5). These findings, together with the fact that hydroxyproline levels in segments proximal and distal to the anastomosis do not change significantly in ileum, clearly demonstrate that, although qualitatively similar processes occur in ileum and colon, the loss of collagen is less extensive and more quickly undone in ileum.

It should be emphasized that Figures 1 and 2 do not necessarily reflect the true course of collagen breakdown. The concentrations are the net results of lysis and synthesis. Jiborn et al (4) have shown that considerable collagen synthesis occurs shortly, meaning 48 h, after construction of colonic anastomosis in the rat. Thus, actual collagen breakdown may be greater than depicted in Figure 1.

Hunt et al (10) have argued that the collagenous equilibrium is most important to the process of colon repair: anastomotic integrity is essentially a race between collagen synthesis and lysis. If synthesis does not overtake lysis, anastomotic leakage occurs. Consequently, control of lysis could be a crucial step in preventing anastomotic dehiscence.

The first step in the breakdown of collagen molecules, cleavage of the triple helix, is performed by the enzyme collagenase. The cellular response after wounding, a sequential appearance of polymorphonuclear neutrophilic leucocytes (granulocytes), macrophages and fibroblasts has been well characterized (2). Microscopic examination of the anastomotic area shows that in our experiments the first

monocytes and fibroblasts only appear 24 h after wounding, when collagen lysis is well under way. Granulocytes are present already after a few hours and these cells are capable of producing a true collagenase (11). While the existence of collagen-bound collagenase has also been demonstrated in animal tissues (12) our results exclude macrophages and fibroblasts as sources of collagenase during the first 24 h after wounding, a period where massive collagen lysis already occurs. Thus, seeking to control collagenolysis after intestinal anastomosis, further research into regulation, and particularly inhibition, of granulocyte and collagen-bound collagenase, if present appears necessary.

Acknowledgements

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Chapter 3

A REPRODUCIBLE MODEL FOR PROTRACTED PERITONITIS IN THE RABBIT

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3.1 Summary

The intra-abdominal implantation of a gelatin capsule containing human fecal material introduces peritonitis in the rabbit. The severity of the disease and the length of survival depend on the volume of the inoculum. A 7-day survival chance varies between 45 and 92%, depending on fecal loading. All rabbits develop intra-abdominal abscesses and in the surviving animals infection persists for at least a week, allowing further study of several aspects of peritonitis.

3.2 Introduction

Peritonitis continues to be a serious disease with high morbidity and mortality. The causes of treatment failure may be divided roughly into two categories: systemic and local. Techniques described for existing experimental models (1) lead to an acute infection with a high mortality in the first 72 h and disappearance of the peritonitis in the surviving animals. These models are well suited to study the systemic effects of peritonitis and the influence of antibiotics and chemotherapy (2).

A major local problem in peritonitis is the tendency of intestinal anastomoses, established in infected surroundings, to leak. This renders elimination of the primary source of infection difficult or even impossible. The crucial phase of healing of the intestinal wound extends over at least a week. Therefore, when studying the influence of infection on the

healing of intestinal anastomoses, an experimental model is needed which combines a persistent intra-abdominal infection with an acceptable mortality. These requirements are not met by existing models.

The present paper describes such a model in the New Zealand White rabbit, which was developed from the findings described by Nichols et al (3) in the rat.

3.3 Materials and methods

3.3.1 Inoculum

Feces from a human volunteer were freshly collected and immediately transferred into an anaerobic chamber. Here, the fecal material was diluted 1 : 4 with Brain Heart Infusion Broth (Difco Labs, Detroit, Michigan, USA), supplemented with glucose and yeast extract, and homogenized. After filtration the suspension was mixed with $BaSO_4$ (10% w/v) to enhance local reaction. The mixture was dispensed into plastic tubes, frozen in liquid nitrogen and stored at $-40^{\circ}C$. Aliquots were diluted tenfold and cultured on Levine EMB agar (Difco Labs), blood-agar (CMSS ovoid) and a medium described by Koopman et al (4), to which 7% sheep blood was added. The latter media were incubated anaerobically for 2 days. After incubation the number of colonies was counted on plates containing between 30 and 300 colonies. The number of anaerobic bacteria (Koopman medium), Enterobacteriaceae (Levine EMB) and aerobic bacteria (blood agar) was calculated from these data. All determinations were performed in duplicate.

3.3.2 Experiment

Sixty-four male New Zealand White rabbits were used, varying in weight between 1.800 and 2.900 g. Surgical procedures were performed in sterile conditions. Rabbits were anesthesized with

an oxygen-nitrous oxide-fluothane mixture. The abdomen was opened through a midline incision of approximately 4 cm. After ligation of the bowel an end-to-end anastomosis was constructed in either ileum (10 cm proximal to the appendix top) or descending colon (3-5 cm above the Douglas pouch) using one layer of interrupted sutures with Prolene^{R} 5 x 0. After establishing the anastomosis, the recently defrozen fecal suspension was imbedded in a gelatin capsule and placed near the anastomosis. Peritoneum and fascia were closed in one layer, with a continuous suture using Vicryl 3 x 0 and the skin was closed with silk 2 x 0. The animals were divided into three groups which received 0.3 ml (n=17), 0.4 ml (n=36) or 0.5 ml (n=11) of the fecal suspension, respectively. At the end of the operation 50 ml physiological saline was injected subcutaneously and this procedure was repeated twice during the next 2 days. The animals had free access to food and water.

In order to examine various anastomotic parameters (not reported here) a number of animals were sacrificed, by means of an overdose of pentothal, on the 3rd and 7th day after operation. The abdomen was opened under sterile conditions and any intraperitoneal fluid or purulent collections were sampled for aerobic and anaerobic cultures. Only qualitative determinations were performed.

3.4 Results

Storage for 3 months under the conditions described did not significantly affect the number of viable aerobic bacteria, anaerobic bacteria and Enterobacteriaceae in the inoculum.

Postoperative weight loss was taken as a parameter of the general condition of the animals and thus as a sign of continuing infection. The mean postoperative loss of weight of the infected animals was compared with that in groups of noninfected rabbits which had the same type of bowel

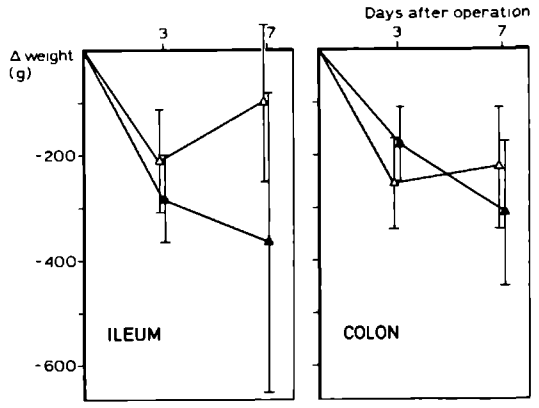

Figure 1:

Effect of infection on weight loss after construction of bowel anastomosis. Average change (g) with SD is depicted at 3 and 7 days after operation with (▲) or without (Δ) induced infection. Number of animals are: with ileal anastomosis, uninfected n=12 (3 days) and n=11 (7 days), infected n=12 3 days and n=9 (7 days); with colonic anastomosis, uninfected n=13 (3 days) and n=14 (7 days), infected n=10 (3 days) and n=8 (7 days).

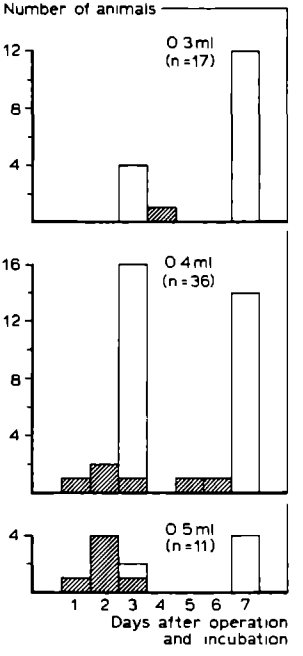

Figure 2:

Effect of different dosage of fecal inoculum on mortality after bowel anastomosis. In each group the number of animals sacrificed is represented (white bars) together with the number of animals dying spontaneously (striped bars).

anastomosis performed (Figure 1). On the 3rd postoperative day all animals, both in the infected and the non-infected group, had lost weight to a similar extent. Loss of weight 7 days after ileal anastomosis was significantly

(0.001 < p < 0.01; Kruskal-Wallis K-sample test) greater in the infected group, as compared to the healthy animals. The level of significance of this difference between infected and non-infected groups was lower (0.05 < p < 0.1) in the rabbits with colonic anastomoses.

Infection was induced by 3 different doses of inoculum (Figure 2):

Dose of 0.3 ml: 1 out of 17 animals in this group died spontaneously on the 4th postoperative day. The 4 animals sacrificed on the 3rd day all displayed a peritoneal fluid collection. The 12 animals sacrificed on the 7th day all showed fibrinous adhesions with abscesses. Aerobes were cultured in 3, anaerobes in 4 and both aerobes and anaerobes in 9 animals.

Dose of 0.4 ml: 6 out of the 36 animals in this group died spontaneously within a week. Initially all rabbits were clearly ill: they refused food, were immobile and produced loose stools. The 16 animals sacrificed on the 3rd day showed a fibrinopurulent peritonitis. Most of the remaining animals had recovered somewhat on the 7th day. At sacrifice all these 14 animals showed encapsulated intraperitoneal abscesses and often a wound abscess as well. Aerobes were cultured in 5, anaerobes in 1 and both aerobes and anaerobes in 24 animals.

Dose of 0.5 ml: 6 out of the 11 animals in this group died within 3 days from a widespread fibrinopurulent peritonitis. Three rabbits which could be sacrificed on the 7th day displayed multiple intra-abdominal abscesses which contained both aerobes and anaerobes.

A statistical comparison (using a generalized Wilcoxon test according to Gehan) of the 3 groups which had received increasing doses of human fecal inoculum showed no significant

(p=0.152) difference in survival rate between the animals receiving 0.3 and 0.4 ml inoculum, respectively. However, survival among the animals which had received 0.5 ml inoculum was significantly shorter than in animals receiving 0.3 ml (p=0.002) and 0.4 ml (p=0.005) inoculum, respectively. The chance for an animal to survive for 7 days after inoculation was calculated as 92% for the 0.3-ml dose, 78% for the 0.4-ml dose and 45% for the 0.5-ml dose.

3.5 Discussion

Preliminary experiments in our laboratory with appendiceal ligation (5) and devascularization procedures (6) resulted in a mortality within 3 days of 50% and disappearance of intra-peritoneal infection in the surviving animals. At sacrifice on the 7th day only fibrinous adhesions were found, but no purulent fluid or abscesses. Techniques based on the introduction of bacterial suspensions into the abdominal cavity (1,7) induce a septic shock rather than a situation comparable to human peritonitis. Undefined material present in sterilized feces (8) and $BaSO_4$ (9,10) may be used to increase local reaction. Introduction of the material by means of a gelatin capsule (9) effectuates a slow release. A mortality of 43% in 7 days and intra-abdominal abscesses in the surviving animals has been reported (9), but in our hands this technique resulted in 100% mortality within 3 days.

The use of human feces (3) in the rabbit has resulted in a reproducible model which suited our purpose. In all sacrificed animals positive cultures were obtained, proving the presence of infection. In addition, the continuing weight loss indicates that the infection continued to influence the general condition of the animals up to the 7th day.

Our results also indicate that severity of infection can be varied by changing the dosage. Presumably, the amount of fecal

material rather than the number of bacteria, which varies relatively little between dosages, determines the degree of peritonitis.

It is noteworthy that quick freezing of the fecal material in liquid nitrogen produced an inoculum that could be stored for at least 3 months without loss of viable bacteria and thus allows reproducible inoculation during a long period of experimentation.

In conclusion, an inoculum produced from human feces induces an intraperitoneal infection and intra-abdominal abscesses in rabbits - a situation closely resembling the local conditions of peritonitis in humans. The early mortality is acceptable and the infection continues for at least a week, allowing further study of several aspects of peritonitis.

Acknowledgements

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3.6 References

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WOUND HEALING IN THE INTESTINAL WALL: EFFECTS OF INFECTION ON EXPERIMENTAL ILEAL AND COLONIC ANASTOMOSES

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4.1 Summary

The healing of both rabbit ileal and colonic infected anastomoses has been investigated. Infection was induced by implanting a capsule with human fecal material in the anastomotic area. Infection did result in lowered bursting pressures, which effect was most pronounced in ileum seven days postoperatively. In general, the average hydroxyproline levels in and around infected anastomoses were lower than the hydroxyproline concentrations measured around non-infected anastomoses. This difference was most significant in the segment proximal to the ileal anastomosis seven days after operation, in the segment distal to the colonic anastomosis three days after operation and in the segment proximal to the colonic anastomosis seven days after operation.

It is concluded that infection interferes with the early stages of the healing sequence in rabbit intestinal anastomoses, profoundly affecting collagen metabolism. Our work does not support recent publications that report an unchanged or even increased wound strength under infected conditions.

4.2 Introduction

The effects of infection on wound healing are still poorly understood. It has been concluded from clinical data that wound dehiscence of abdominal wounds is more likely to occur if infection is present (1,2). However, experimental studies

have produced conflicting results. In addition to a decreased wound strength in infected abdominal wounds (3,4), no significant deleterious effects of infection (5) or even increased tensile strength in laparotomy wounds after infection (6,7) have been reported.

The role of infection in disruption of intestinal anastomoses is also a matter of some doubt. Irvin and Goligher (8) have found no evidence that peritoneal sepsis contributes toward anastomotic complications, while Schrock et al (9) concluded that infection strongly favours anastomotic leakage. Still, the general attitude appears to be that the presence of local infection is a strong reason for staging of intestinal resection (10,11).

There are very few data available on the effects of infection on healing of experimental intestinal anastomoses. Studies on the underlying process in wound healing should involve investigations into the behavior of collagen because of the multitude of cardinal roles played in the repair process by this connective tissue macromolecule (12) and because the submucosal collagen ultimately provides the intestinal wall with strength to withstand intraluminal pressure (13). Indeed, some evidence is available that peritoneal infection affects collagen metabolism in colonic anastomoses (14,15). However, much further research is needed to understand, and eventually control, the process by which infection interferes with anastomotic healing. The present paper reports on the effects of induced peritonitis on mechanical strength and collagen content of ileal and colonic anastomoses in the rabbit.

4.3 Methods

This study involved 95 male New Zealand White rabbits whose weight at operation varied between 1550 and 2900 grams. Surgical procedures were described in a preceding paper (16).

Briefly, in each rabbit two anastomoses were constructed in either ileum or colon. In ileum, the first anastomosis (end-to-end using one layer of interrupted inverting sutures with Prolene® 5 x 0) was placed 10 cm proximal to the appendix tip and the second anastomosis approximately 20 cm in the proximal direction. In colon, the lower anastomosis was constructed 3 to 5 cm above the Douglas pouch and the second anastomosis 3 cm under the splenic flexure. During operation a control segment was removed for determination of bursting pressure and hydroxyproline content. Postoperatively, the animals were fed ad libitum. After three or seven days the rabbits were sacrificed and a 5-cm segment containing the more distal anastomosis was removed for determination of bursting pressure and hydroxyproline content. The more proximal anastomosis was used for examination of vascularization and histology (results not reported in this paper).

Peritonitis was induced by leaving a gelatin capsule with human fecal material near the anastomosis during operation. This procedure, which is described more extensively elsewhere (17), yields an acceptable short-term mortality, eight of 64 animals dying spontaneously within 48 hours, thus allowing enough animals to survive for three and seven days, respectively. All animals treated this way develop intra-abdominal abscesses and positive bacterial counts of peritoneal contents: in the surviving animals infection persisted for at least a week.

Bursting pressure was determined by infusion of an aqueous solution of methylene blue into the segment, which had been connected to an infusion pump and a manometer (16).

Hydroxyproline was determined in the control segment and in three 1-cm samples from the anastomotic segment, one containing the anastomosis and the other two representing the adjacent proximal and distal parts, respectively. All samples were

Table 1: *Pre-operative weight and per-operative intestinal bursting pressure and hydroxyproline content in the various experimental groups.*

	experimental group	number of rabbits	weight	bursting pressure (mm Hg)	hydroxyproline (µg/mg dry weight)
ILEUM	3D	14	2410 ± 219	172 ± 43	8.9 ± 0.8
	3D-infected	12	2444 ± 226	162 ± 30	10.1 ± 1.2)*
	7D	14	2311 ± 358	189 ± 33	8.7 ± 1.2
	7D-infected	9	2316 ± 227	152 ± 18)**	8.9 ± 0.9
COLON	3D	14	2307 ± 249	81 ± 23	12.9 ± 1.6
	3D-infected	10	2407 ± 290	74 ± 9	14.1 ± 1.8
	7D	14	2380 ± 265	81 ± 13	13.0 ± 1.7
	7D-infected	8	2253 ± 349	82 ± 7	13.3 ± 1.0

Mean values are given together with standard deviations. Significant differences occurring within either the ileum-operated or the colon-operated groups are indicated (* $0.01 < p < 0.05$, ** $0.001 < p < 0.01$; Kruskal-Wallis K-sample test).

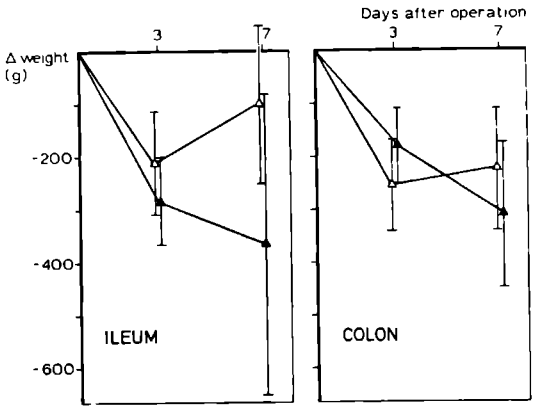

Figure 1:
Loss of weight after construction of intestinal anastomosis. Results are depicted as average change (g) with standard deviation at three and seven days after operation with (▲) or without (Δ) induced infection.

frozen immediately in liquid nitrogen. Thereafter, samples were pulverized in a Braun microdismembrator, lyophilized and stored at -30°C. Hydroxyproline was measured essentially according to Prockop and Udenfriend (16,18).

4.4 Results

The rabbits were divided into eight groups, four with ileal and four with colonic anastomoses, which were sacrificed three or seven days after operation. In half of the groups peritonitis was induced. Table 1 gives the number of animals in each group. Average weight at operation was the same in all groups.

Figure 1 shows that operation induced a significant loss of weight, which was more extensive if infection was present. Three days postoperatively all animals had lost weight. Infection delayed subsequent recovery of weight, particularly after ileal anastomosis. Loss of weight seven days after ileal anastomosis was significantly ($0.001<p<0.01$; Kruskal-Wallis K-sample test) greater in the infected group, as compared with the healthy animals. The level of significance of this difference between infected and noninfected groups was lower ($0.05<p<0.1$) in the rabbits with colonic anastomoses.

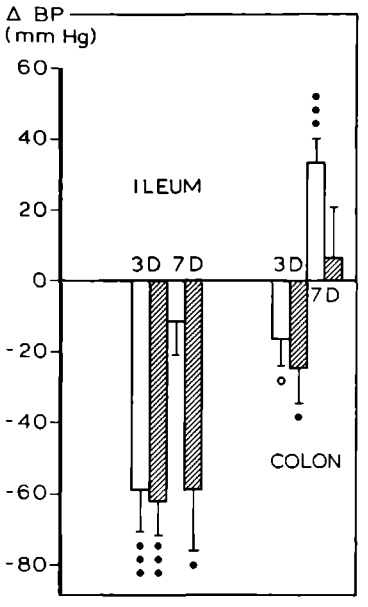

Figure 2:

Changes in intestinal bursting pressure after construction of an intestinal anastomosis. Bursting pressures of the intestinal segment around the anastomosis are measured three (3D) and 7 (7D) days after operation and compared with pre-operative bursting pressures. Shaded columns represent infected groups, white columns non-infected groups. Results are expressed as average change (mm Hg) with standard deviation. Levels of significance are calculated by means of a paired sample t-test and represented in the following way: o: $0.05<p<0.1$; ●: $0.01<p<0.05$; ●●●: $p<0.001$.

4.4.1 Bursting pressure

The mean bursting pressures of unwounded intestine in the various experimental groups are given in Table 1. No significant differences were found between the groups that were to receive a colonic anastomosis. Among the groups that were due to receive an ileal anastomosis, basal values in the 7D group were significantly higher than those observed in the 7D-infected group. Also, bursting pressures in ileum were clearly higher than bursting pressures in colon.

Absolute changes in intestinal bursting pressures are depicted in Figure 2. Construction of an anastomosis led to a loss of strength, which seemed more pronounced in ileum. Seven days after operation, bursting pressure around uninfected ileal anastomoses did not differ significantly from the value obtained from unwounded tissue, while infected anastomoses did exhibit a significantly lower bursting pressure (compared with

Table 2: *Bursting place in intestinal segments containing an anastomosis*

	experimental group	bursting within anastomosis	bursting elsewhere	level of significance
ILEUM	3D	10	4	ns
	3D-infected	10	2	
	7D	0	14	$0.01<p<0.05$
	7D-infected	3	6	
COLON	3D	7	7	ns
	3D-infected	8	2	
	7D	0	14	ns
	7D-infected	2	6	

The effects of infection on the frequency of perforations occurring within the anastomotic area is shown. Levels of significance of the difference between infected and non-infected groups are calculated with Fishers exact test for the two times two table.

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values obtained at operation). A direct comparison of the decrease in bursting pressure measured in infected (average: 59 mm Hg) and noninfected (average: 11 mm Hg) animals seven days postoperatively indicates a significantly greater ($0.01 <p<0.05$; Kruskal-Wallis K-sample test) loss of bursting strength in infected anastomotic segments.

In colon, three days postoperatively, a mean loss of bursting pressure of 16 mm Hg occurred in healthy tissue, which decrease was almost significant ($0.05<p<0.1$). Also, in non-infected animals, seven days after operation the anastomotic area had grown much stronger than the unwounded tissue, while in infected rabbits values at this stage were similar to those observed peroperatively. Thus, anastomoses in infected areas certainly appeared to be weaker than those placed in noninfected

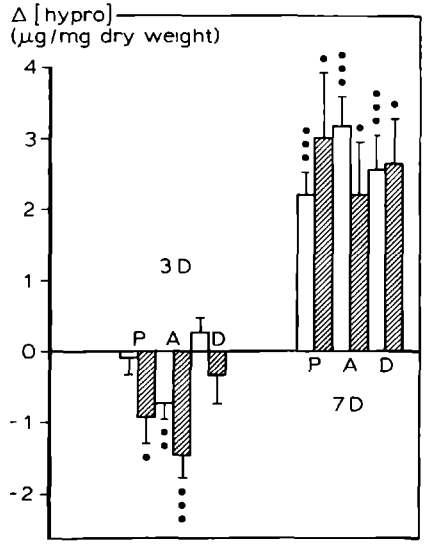

Figure 3:

Changes in hydroxyproline content of intestinal segments after construction of an ileal anastomosis. Hydroxyproline is measured immediately around the anastomosis (A) and in a proximal (P) and a distal (D) segment and compared with the preoperative value. Shaded columns represent infected groups, white columns non-infected groups. Results are expressed as average change (µg/mg dry weight) with standard deviation. Levels of significance for the differences observed are calculated by means of a paired sample t-test and represented in the following way: ●: $0.01<p<0.05$; ●●: $0.001<p<0.01$; ●●●: $p<0.001$.

tissue. The temporary loss of strength induced by anastomotic construction is also illustrated by the frequency of perforations occurring in the anastomosis as compared with perforations occurring outside the proper healing area (Table 2). In noninfected tissue, three days postoperatively the intestine frequently perforated in the anastomosis; seven days after operation the strength of the anastomosis was always greater than that of the adjacent segment. Comparing infected and noninfected groups, the frequency of perforations occurring in the anastomotic area was always higher in the presence of infection. However, this difference was only significant in the ileum-operated animals seven days after operation.

4.4.2 Hydroxyproline content

Peroperative hydroxyproline levels in the experimental groups are shown in Table 1. Hydroxyproline concentrations in colon were higher than in ileum. Average hydroxyproline in the ileum 3D-infected group was significantly elevated when compared with the 3D group.

Figure 3 shows the changes in hydroxyproline concentration observed around infected and noninfected ileal anastomoses. Three days after construction the hydroxyproline level in the anastomotic segment was lowered significantly ($0.001 < p < 0.01$), while it remained unchanged in both proximal and distal segments. If fecal material was implanted near the anastomosis during operation, loss of hydroxyproline was more extensive. The change in the anastomotic area was even more distinctive ($p < 0.001$) and was accompanied by a significant decrease in hydroxyproline level in the proximal segment. Direct comparison of the changes encountered proximal to the anastomosis in infected and noninfected animals showed a significantly ($0.01 < p < 0.05$; Wilcoxon two-sample test) greater change in infected rabbits. Seven days after operation in the absence of infection, the hydroxyproline content of all segments was strongly ($p < 0.001$) enhanced as compared with the level in unwounded tissue. A similar increase was observed in the presence of infection, only the level of significance for the difference between postoperative and peroperative concentrations was lower ($0.01 < p < 0.05$).

Colonic anastomosis in healthy rabbits led to a great loss of hydroxyproline in the anastomotic and proximal segments after three days (Figure 4). No change was observed in the distal part. In infected animals changes were more extensive. The average decrease in the anastomotic area was approximately 20 per cent higher than in noninfected conditions, which difference proved to be nearly significant ($0.05 < p < 0.1$; Wilcoxon two-sample test). Moreover, the distal segment now also displayed a very significant loss of hydroxyproline. This effect was also significantly ($0.001 < p < 0.01$; Wilcoxon two-sample test) greater than that observed in noninfected animals. After seven days, hydroxyproline levels in the three intestinal segments were back to starting values, at least under noninfected conditions. In the presence of infection, both proximal and anastomotic segments still showed a

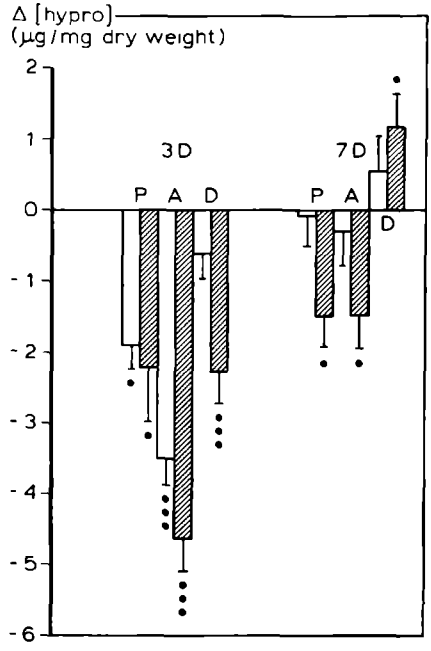

Figure 4:

Changes in hydroxyproline content of intestinal segments after construction of a colonic anastomosis. Hydroxyproline is measured immediately around the anastomosis (A) and in a proximal (P) and a distal (D) segment and compared with the pre-operative value. Shaded columns represent infected groups, white columns non-infected groups. Results are expressed as average change (µg/mg dry weight) with standard deviation. Levels of significance for the differences observed are calculated by means of a paired sample t-test and represented in the following way. ●. *$0.01<p<0.05$;* ●●●: *$p<0.001$.*

significant lowering of hydroxyproline while, surprisingly, the hydroxyproline concentration in the distal segment was elevated. Direct comparison of the changes in infected and noninfected animals showed a significant ($0.01<p<0.05$; Wilcoxon two-sample test) difference in the area proximal to the anastomosis. Thus, on the whole, loss of hydroxyproline proved to be more extensive in the presence of infection.

4.5 Discussion

Experimental evidence supporting the general opinion of surgeons that infection impairs wound healing is still scarce and conflicting. It is particularly intriguing that some authors have demonstrated repeatedly that bacterial infection may even lead to an increased wound strength of rat abdominal incisions. This acceleration of wound healing has also been reported for skin incisions in rats (19).

The effect of infection on the bursting pressure of intestinal anastomoses has not been documented before. Hawley (20) mentioned that the bursting pressure of infected colonic anastomoses was lower than in noninfected controls on the seventh, but not on the third, postoperative day. However, he did not supply any experimental data. Our results (Figure 2, Table 2) certainly indicate a deleterious effect of infection on the strength of intestinal anastomoses, which was most pronounced in ileum seven days after operation, and thus support the hypothesis that infection adversely affects wound healing.

On the molecular level, the process of wound healing is often examined by measuring the changes in tissue collagen content. Collagen plays a central role in the healing sequence (12) and newly formed collagen fibers are primarily responsible for the development of wound strength. However, a direct correlation between lowered wound strength, as measured by physical means, and decreased wound collagen content, as measured by total hydroxyproline, has not been established in infected wounds. A lower tensile strength together with increased hydroxyproline content have been reported (4), as well as decreased wound strength together with unchanged hydroxyproline levels (3). While our results have not shown a direct correlation between bursting pressure and hydroxyproline content in the various experimental groups, the increased loss of bursting strength in the presence of infection appears to be attended with enhanced loss of hydroxyproline.

Changes in collagen of infected intestinal anastomoses have been reported before only in colon. Injection of a fecal suspension into the wall of the sigmoid colon of dogs resulted, up until 14 days after operation, in significantly lower hydroxyproline levels in the anastomotic area, compared with noninfected controls (14). Rat colonic anastomoses

infected with powdered autoclaved rat feces showed only a significant reduction in the amount of salt-soluble hydroxyproline, which constituted less than 1 per cent of total tissue hydroxyproline, on the third postoperative day compared with anastomoses of the control group (15). Our results with colonic anastomoses (Figure 4) indicate pronounced effects of infection on total hydroxyproline. Three days postoperatively mean hydroxyproline content was lower in infected than in noninfected intestine in all three segments investigated. The difference was most significant in the area distal to the anastomosis, indicating that infection does not affect the proper healing area only. This enhanced loss of collagen was also present seven days after operation.

Healing of ileal anastomoses has not been documented before. We have found that this process is also characterized by a transient loss of collagen, though less extensive and more quickly compensated than in colonic anastomoses (16). Again, infection seemed to result in a fairly consistent decreased collagen content, as compared with noninfected controls, particularly after three days (Figure 3). Here, the difference was most pronounced in the area proximal to the anastomosis.

If one considers the total of the results presented here, the general picture that emerges is one of an impaired wound healing induced by infection. Recovery of both bursting pressure and collagen content appear retarded under infected conditions. It remains to be seen whether infection enhances collagen lysis or inhibits the onset of collagen synthesis; the net result of these processes is thought to determine anastomotic integrity (21). Further research in our laboratory aims to define the factors responsible for inducing the massive collagen lysis that starts within hours after construction of an intestinal anastomosis (unpublished observations). Reduction of the enhanced loss of hydroxyproline, presently found in

infected conditions, may lead to improved healing of infected anastomoses.

Acknowledgments

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HISTOLOGICAL FEATURES OF WOUND REPAIR: A COMPARISON BETWEEN EXPERIMENTAL ILEAL AND COLONIC ANASTOMOSES

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5.1 Summary

Various histological parameters of wound repair have been studied in intestinal anastomoses in the rabbit in order to compare the healing processes in ileum and colon. The sequentional appearance of granulocytes, macrophages and fibroblasts has been studied from 3 hours to 7 days after operation. Also, degree of necrosis, formation of capillaries and mucosal repair were analyzed semi-quantitatively.

Significant differences were observed between ileal and colonic anastomoses with respect to occurrence of granulocytes, necrosis and mucosal repair, particularly 7 days after operation. At that time, granulocytes and necrosis were virtually absent from ileal anastomoses, while mucosal integrity was restored in the majority of cases examined. In contrast, granulocytes and necrosis were still abundantly present in colonic anastomoses and mucosal repair was poor. These results support biochemical evidence that wounds in the ileum heal more rapidly than wounds in the colon. Possibly, the sustained presence of granulocytes, which are a potential source of collagenase activity, is important in this respect. Investigation of the same features of intestinal wound healing in rabbits with peritonitis induced by infection showed similar results and no differences were found between infected and non-infected animals.

5.2 Introduction

Failure of large bowel anastomoses is the major complication of colonic surgery. Reported rates of clinical leakage vary but may be as high as 30% (1,2). The associated morbidity and mortality fully warrant research into its causes and possible prevention. Studies of biochemical processes involved in the healing of experimental colonic anastomoses demonstrate a massive lysis of collagen during the first days after surgery (3). It has been postulated that the 'collagenous equilibrium' is critical for the integrity of the anastomosis (4): leakage occurs if synthesis does not overtake lysis in time.

Since leakage of ileal anastomoses does not appear to be a significant feature of surgery of the small bowel, we decided to compare the healing processes in ileal and colonic anastomoses in the rabbit. It appears that though ileal anastomoses also lose collagen during the first days after surgery, the loss is less extensive and more rapidly restored than in colonic anastomoses (5).

The presence of an established local infection is thought to favour anastomotic leakage (3). We have shown that infection impairs healing of experimental intestinal anastomoses, retarding recovery of bursting pressure and collagen content (6). The present study was undertaken to compare the histological sequence of events in both ileal and colonic anastomoses and to assess the effect of infection.

5.3 Materials and methods

The experiments were done on 112 male New Zealand White rabbits varying in weight between 1550 and 3000 g; 58 animals were subjected to ileal anastomosis and 54 to colonic anastomosis. Surgical procedures have been described else-

where (5). In 16 animals with ileal anastomoses and 17 animals with colonic anastomoses peritonitis was induced per-operatively by implantation of capsules with human feces next to the suture line (7). The organismal content of all capsules was equal. Post-operatively the animals were fed ad libitum. The animals with ileal anastomoses were sacrificed by means of an overdose of penthotal, after 3h (n=5), 12h (n=6), 1 day (n=3), 2 days (n=3), 3 days (n=12, uninfected; n=9, infected) and 7 days (n=13, uninfected, n=7, infected). Rabbits with a colonic anastomosis were sacrificed after 3h (n=4), 12h (n=2), 1 day (n=3), 2 days (n=3), 3 days (n=11, uninfected; n=8, infected) and 7 days (n=14, uninfected; n=9, infected).

The anastomotic segments were removed and collected in 4% (w/v) formaldehyde. Samples were successively dehydrated with acetone, methyl benzoate and toluene and embedded in paraffin; 6µ sections were stained with hematoxylin-eosin. The presence of different types of cells, the ingrowth of newly formed blood vessels, the healing of the mucosa and the degree of necrosis were recorded semi-quantitatively. Examination of all sections was performed by one person, who was unaware of the experimental group from which the specimen originated.

5.4 Results

The appearance of various types of cells in the wound area during the first 48h after surgery was similar in both ileal and colonic anastomoses (Table 1). Granulocytes were found already after 3h; their number reached a maximum after 12-24h. Monocytes, regarded as the precursors of macrophages, and fibroblasts could be observed at 24h after operation. Both fibroblasts and macrophages remained abundantly present in the majority of 3-day and 7-day anastomoses from both parts of the gut (Table 2). Granulocytes were also found almost invariably after 3 days. However, a very significant

Table 1: *Occurrence of different types of cells in sections of intestinal anastomoses during the first 48 hours after operation. Results are similar for ileal and colonic anastomoses.*

hours after operation	n (ileum, colon)	granulocytes	monocytes/ macrophages	fibroblasts
3	9 (5,4)	+	-	-
12	8 (6,2)	++	-	-
24	6 (3,3)	++	±	+
48	6 (3,3)	+	+	++

\- absent
± occasional cell present
\+ present
++ abundantly present

---

difference between ileal and colonic anastomoses became apparent after 7 days. While in ileum granulocytes had mostly disappeared from the wound area (absent in 9 out of 13 anastomoses), they could still be observed in great numbers in 12 out of the 14 colonic anastomoses examined.

Further differences were found with respect to removal of necrosis and degree of mucosal repair. At 3 days, the necrosis had been cleared away completely in 3 of 12 cases of ileal anastomoses and the gap in the mucosa had been adequately restored in 8 of 12. Neither phenomenon was observed in any of the colonic anastomoses. At 7 days, this difference was even more evident, necrosis still being abundantly present in 11 out of 14 cases in colon and in only 1 out of 13 cases in ileum. The difference in mucosal repair was slightly less spectacular though still highly significant: complete repair in 8 out of 13 ileal anastomoses and in none out of 14 colonic anastomoses. These processes

Table 2: *Various features of wound repair in intestinal anastomoses as observed microscopically 3 and 7 days after operation. For each parameter, differences between groups were tested for significance by Fishers 2-sided exact test for 2x2 tables. Significant differences between ileum and colon are denoted (*) next to the columns representing observations on colon. Likewise, significant differences between 3-day and 7-day anastomoses are denoted (o) next to the column representing observations on ileum 7-days. (*) $0.05 < p \leq 0.1$; oo/** $0.001 < p \leq 0.01$; *** $p \leq 0.001$.*

	Ileum 3 days (n=12)			Colon 3 days (n=11)			Ileum 7 days (n=13)			Colon 7 days (n=14)		
	+	±	-	+	±	-	+	±	-	+	±	-
Necrosis	6	3	3	10	1	0(*)	1	0	12oo	11	2	1***
Granulocytes	9	1	2	11	0	0	2	2	9oo	12	2	0***
Macrophages	10	1	1	10	1	0	11	2	0	11	3	0
Fibroblasts	9	2	1	9	0	2	13	0	0	13	0	1
Capillaries	8	0	4	6	2	3	12	0	1	10	3	1
Mucosal repair	3	1	8	0	0	11	8	0	5	0	3	11**

\+ abundantly present

± occasionally present

\- absent

Figure 1: *Accurately adapted 3-days old ileal anastomosis. Only slight necrosis of the inverted mucosa. Abundant ingrowth of fibroblasts and capillaries into the gap of muscular layer. At the bottom remnants of the suturing material. H.E. 30 x.*

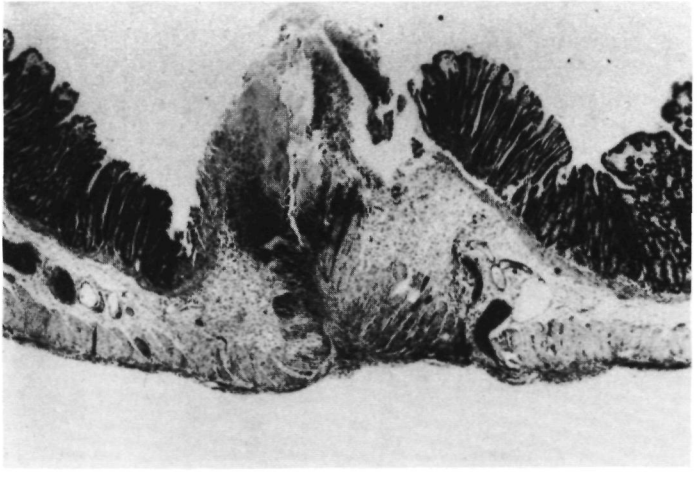

Figure 2: *Three days old colonic anastomosis: massive necrosis of inverted mucosal and muscular layer covers the bottom of an extended defect. Only slight edema and cellular infiltrate in submucosa and peritoneal surface. H.E. 30 x.*

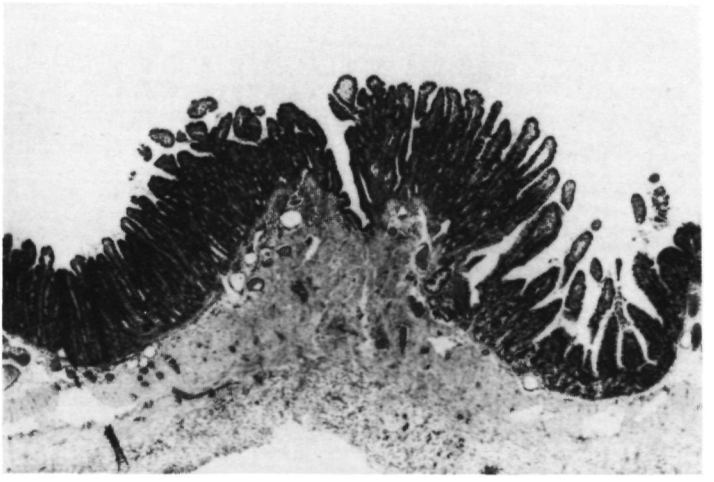

Figure 3: *Completely regenerated mucosal epithelial layer in 7-days old ileal anastomosis. Closely approaching muscular layers. Only slightly thickened peritoneal covering. H.E. 30 x.*

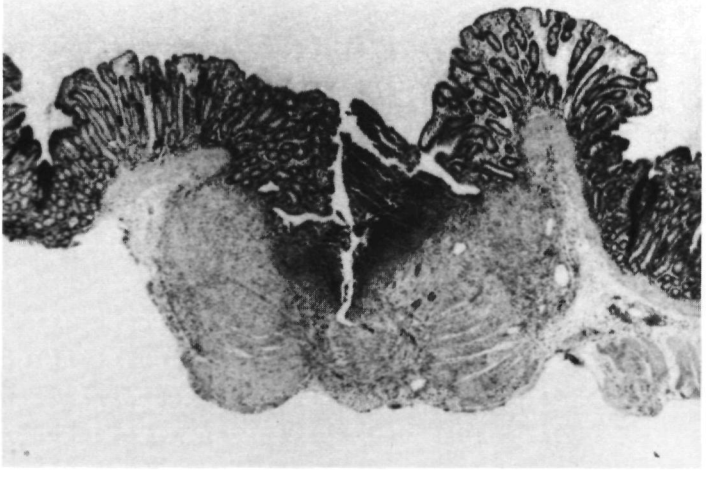

Figure 4: *Seven days old colonic anastomosis: persisting necrosis and forming of a fissure penetrating the granulation tissue joining the adjusted muscular layer. Only slight peritoneal thickening. H.E. 30 x.*

are illustrated in Figures 1 to 4, which show typical examples of both ileal and colonic anastomoses 3 and 7 days after operation. Incomplete mucosal repair and necrosis were apparent in colon after 7 days (Figure 4), while at this time the ileal anastomosis had healed completely (Figure 3). Preliminary angiographic studies on the degree of vascularisation in ileal and colonic anastomoses indicate a similar development of vascularity in both types of anastomoses.

In rabbits where peritonitis was induced by the introduction of human feces, the presence of peritonitis was confirmed after study of the microscopic slides. A notable feature was that the inflammatory reaction seemed to be confined to the serosal covering of the bowel and to the immediate vicinity of the sutures. Transmural progression of the inflammation was not observed, possibly because of the quick and efficient sealing of the serosa at the anastomosis.

The presence of peritonitis did not affect time of appearance, degree or duration of the various features of wound healing studied (Table 3). The differences between ileum and colon which were observed in the absence of infection were found again in the presence of peritonitis. The number of rabbits in each infected group was smaller than in the corresponding non-infected group, because of the relatively high mortality rate after induction of peritonitis (7) and the statistical significance of these differences remained less pronounced.

5.5 Discussion

In the process of wound healing several cell types appear sequentially in a regular pattern (8). Thrombin activation, initiated by thrombocytes, produces fibrin from fibrinogen. Fibrin and the activated complement system induce a chemotactic stimulus leading to the appearance of granulocytes in the wound (9). Thereafter, the first monocytes appear. These are

Table 3: *Various features of wound repair in infected intestinal anastomoses as observed microscopically 3 and 7 days after operation. For each parameter, differences between groups were tested for significance by Fishers 2-sided exact test for 2x2 tables. Significant differences between ileum and colon are denoted (x) next to the columns representing observations on colon. Likewise, significant differences between 3-day and 7-day anastomoses are denoted (o) next to the columns representing observations at 7 days. (x) $0.05 < p \leq 0.1$; o $0.01 < p \leq 0.05$.*

	Ileum 3 days (n=9)			Colon 3 days (n=8)			Ileum 7 days (n=7)			Colon 7 days (n=9)		
	+	±	-	+	±	-	+	±	-	+	±	-
Necrosis	7	0	2	8	0	0	1	0	6^{o}	6	0	3$^{(x)}$
Granulocytes	6	2	1	8	0	0	2	0	5	6	1	2
Macrophages	7	2	0	8	0	0	7	0	0	9	0	0
Fibroblasts	5	1	3	2	3	3	7	0	0	8	1	0^{o}
Capillaries	7	1	1	4	2	2	6	0	1	7	2	0
Mucosal repair	4	0	5	0	0	8$^{(x)}$	5	0	2	2	0	7

\+ abundantly present

± occasionally present

\- absent

regarded as precursors of macrophages. The latter are thought to be important for the removal of necrosis and also stimulate angiogenesis and migration and proliferation of fibroblasts. Thus, the macrophage has a central role in wound repair (10). The ground substance and collagen produced by the fibroblasts lead to the final phase of wound healing, the formation of fibrous tissue. The various features of wound repair observed by us in healing anastomoses of the small and large bowel are similar to this pattern.

After establishment of an intestinal anastomosis the balance between synthesis and breakdown of collagen is lost temporarily. The result is a markedly lowered collagen content (3), which is more extensive and less rapidly restored in colon than in ileum (5). The main feature of the histological findings presented here is the different behaviour of the granulocytes, which have nearly disappeared from the ileal anastomoses 7 days after surgery, while at this time still being abundantly present in almost all colonic anastomoses examined. Granulocytes are known as a potential source of collagenase (11), which is essential to breakdown of collagen fibers. It is tempting to speculate that the prolonged presence of collagenase-producing granulocytes is the cause of the demonstrated greater loss of collagen, and consequently poorer repair, in colonic anastomoses as compared to ileal anastomoses. Still, the absolute level of collagen is the sum of lysis and synthesis and at present it cannot be ruled out that colonic anastomoses show a delayed synthesis.

One might suggest that a difference in vascularity accounts for the differences in the rates of phagocytosis and net collagen accretion. However, our preliminary experiments indicate equal vascularisation of ileal and colonic anastomoses. Furthermore, Shikata et al (12) found no differences between ileum and colon regarding local blood flow, as measured on either the mesenteric, middle or antimesenteric

side. In fact, microcirculation in the wall of the colon in devascularised segments was better than that of the small intestine. Therefore, we do not believe that differences in blood supply can explain the superior healing of ileal anastomoses.

The presence of infection is considered to be a contributing factor to anastomotic dehiscence (1). It has been shown that anastomoses which have to heal in infected surroundings display a lower bursting pressure and a lowered collagen content than anastomoses in non-infected areas (6,13). Hawley (13) has attributed this phenomenon to an increased collagenase activity resulting from infection. The histological examination now reported shows no differences in the healing sequence of infected and non-infected anastomoses: delayed healing as indicated by retarded restoration of pre-operative bursting pressure and collagen content remains invisible at the light-microscopic level. Infection did not affect rate of appearance and disappearance of granulocytes from either type of intestinal anastomosis. However, these results are only semi-quantitative and since no data are available yet on granulocyte -collagenase- activity, they cannot be taken as evidence against a hypothesis that granulocyte collagenase is responsible for the lowered collagen content around infected anastomoses.

In conclusion, it may be stated that - on the microscopic level - significant differences in the rate of wound healing are apparent between experimental ileal and colonic anastomoses, but not between infected and non-infected anastomoses.

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Chapter 6

ANASTOMOTIC INSUFFICIENCY IN SMALL BOWEL SURGERY

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Submitted

6.1 Summary

In contrast to the abundant information available on the frequency and causes of disturbed healing of colonic anastomoses, there is a paucity of this kind of data with regard to anastomoses of the small bowel. A retrospective study was performed covering the years 1977 through 1981. In this time span 234 small bowel anastomoses were constructed in 143 patients. Anastomotic leakage was seen in 8 patients (3,4%) and fistula formation in 9 patients (3,8%): a total rate of disturbed healing of small bowel anastomoses of 7,3%.

Risk factors studied were intraabdominal infection, vascular compromise and actinic damage. Cases where none of these conditions applied were designated as 'clean'. The rate of leakage in clean cases was 0,8% and is substantially lower than the rate of leakage of colonic anastomoses in comparable patients. In the presence of risk factors the leakage rate was: infection 14,8%; vascular compromise 7,7% and actinic damage 28,6%. The last two groups were too small to allow statistical evaluation. The difference between clean cases and infected cases was statistically significant.

It is concluded that basic conditions in the small bowel are more favourable to sound healing of anastomoses, but that this advantage is annuled by infection.

6.2 Introduction

Anastomotic insufficiency constitutes one of the main hazards of gastrointestinal surgery. About 33% of the mortality after bowel resection is due to this complication and those patients who recover do so after a prolonged illness.

Anastomotic leakage as a complication of colonic surgery has received ample attention in the literature. A number of factors contributing to the development of leakage in colonic anastomoses have emerged (1,2). Measures to avoid this complication (e.g. resection without immediate anastomosis) or to lessen the consequences (e.g. a protective enterostomy) are current surgical practice although the rationale of some of these measures is still open to discussion (3).

In contrast, data on small bowel anastomoses are practically non-existent. A computer-assisted (Medlars) search of the literature produced only two papers (4,5), which demonstrate the serious nature of small bowel anastomotic insufficiency but do not provide data on the incidence of the complication. There is abundant information on the course, treatment and prognosis of small intestinal fistulas (e.g. 6,7,8). These papers give some information about possible contributing factors, but again the incidence of this complication cannot be derived from the communications.

Because small bowel anastomotic leakage is as dangerous as colonic leakage, prevention has the same importance in both. As a first step to fill the gap in our knowledge we have reviewed our experience and present these retrospective data as a base-line for further studies.

6.3 Patients and methods

In the five years 1977 through 1981 234 small bowel

anastomoses were performed in 143 adult patients. Operations were performed by staff surgeons or by senior residents under direct supervision of staff surgeons. Anastomoses were made with an outer layer of interrupted inverting sutures and an inner continuous layer, both with synthetic absorbable material (Vicryl^{R}). In cases of mesenteric vascular occlusion a second look procedure was used. Small bowel resections in the course of re-operations for postoperative peritonitis were not included. When local conditions were judged to be highly unfavorable (diffuse primary peritonitis or large abscesses) resection without anastomosis was chosen as a first step. In the period under review, this was done in 16 patients not included in this report, but indicated in Table 1.

For the purpose of this study, patients were divided into the following risk groups:
- 'vascular': patients in whom a devitalised segment of bowel was resected;
- 'inflammatory': patients with either an inflammatory process in the bowel, a positive culture of peritoneal fluid or a localised abscess;
- 'irradiated': patients operated upon after abdominal radiotherapy;
- 'clean': where none of the above was applicable, e.g. trauma, bleeding or obstruction.

Complications were divided into anastomotic leakage and fistula. In leakage there was free spread of intestinal contents in the abdominal cavity with appropriate clinical symptoms. Fistula was a localised process with leakage of intestinal contents to the outside, usually through the surgical wound.

Subclinical defects in the integrity of an anastomosis are frequent in lower anterior resection. They can easily be

Table 1

Clean		Inflammatory	
Age	15-88 yrs	Age	16-71 yrs
Mean	49 yrs	Mean	41 yrs
Trauma	5	Crohn's disease	35
Metastatic cancer	22	Fistula	11
Morbid obesity	6	Diverticulitis	11
Small intestinal tumors	8	Perforation	3
Angiodysplasia	5	Miscellaneous	4
Meckel's diverticulum	4		64
Miscellaneous	10		
	60		

Vascular		Irradiated	
Age	22-85 yrs	Age	36-72 yrs
Mean	53 yrs	Mean	60 yrs
Incarcerated inguinal hernia	6	Carcinoma of	
Mesenteric vascular occlusion	10	bladder	4
Volvulus	3	ovary	1
Strangulation	10	kidney	1
	29		6

Split stoma: Clean 3 (2 trauma, 1 metastatic cancer)
Inflammatory 9 (8 Crohn's disease, 1 miscellaneous)
Vascular 3 (1 incarcerated hernia, 2 strangulation)
Irradiated 1 (carcinoma of bladder)

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detected by contrast enema. This is not feasible in the case of small bowel anastomosis and therefore subclinical defects are not included in this report.

Table 2

	Clean	Inflammatory	Vascular	Irradiated
Number of patients	57	55	26	5
Number of anastomoses	120	81	26	7
Anastomotic leakage	1 (0,8%)	4 (4,9%)	2 (7,7%)	1 (14,3%)
Fistula	0	8 (9,9%)	0	1 (14,3%)
Total number of complications	1 (0,8%)	12 (14,8%)	2 (7,7%)	2 (28,6%)

Table 3

	Jejuno-jejunal	Ileo-ileal	Ileo-colic	Local excision
Number of anastomoses	32	99	51	52
Anastomotic leakage	0	5 (5%)	3 (5,8%)	0
Fistula	0	8 (8%)	1 (1,9%)	0
Total	0	13 (13%)	4 (7,8%)	0

6.4 Results

Operations for clean conditions and for inflammatory conditions made up the bulk of the material, accounting for 78% of patients and 86% of anastomoses (Table 2).

The groups of patients with vascular conditions and actinic lesions proved to be too small to allow for statistical evaluation. The difference between the clean operations and the operations for inflammatory conditions was significant ($p=0.014$). The overall rate of anastomotic leakage was 8 of 234 anastomoses, i.e. 3,4%. The rate of fistula formation was 9/234, i.e. 3,8%. Total rate of disturbed healing of small bowel anastomoses was 17/234, i.e 7,3%. Fistula formation was seen nearly exclusively in the inflammatory group: four in patients with Crohn's disease, two in patients with sigmoid diverticulitis and two were recurrences after re-operation in patients referred with persisting fistulas. With regard to localisation of the anastomoses (Table 3) the jejuno-jejunostomies seem to carry less risk of complications than anastomoses lower down, but the difference was statistically not significant.

6.5 Discussion

The first conclusion is that under 'clean' conditions disturbed healing of small bowel anastomoses rarely occurs. 'Clean' conditions in our patients exclude overt infection, disturbed circulation and actinic damage, factors known to predispose to anastomotic leakage in colonic anastomoses (1). But other known factors such as age, malnutrition and obstruction are not excluded. Indeed, in an appreciable number of patients in this category the anastomosis was performed for obstructing metastatic cancer, which conceivably combines obstruction, advanced age, some degree of mal-nutrition and bacterial overgrowth. Therefore, this category

probably can be compared with patients undergoing surgery of the large bowel for cancer.

Many published series can not be used for this comparison, because they vary widely in exactness of definitions and in composition of the series with regard to tumor localisation, age and other aggravating factors. Probably the fairest comparison is with elective resection for intra-peritoneal tumors. Three papers give these figures (1,9,10) as resp. 3,4%, 3,1% and 3,7%. It would thus seem that with a leakage rate of 0,8% small bowel anastomoses are less prone to leakage than large bowel anastomoses. This confirms a clinical impression and may be the reason why small bowel anastomosis has received so much less attention in the literature than large bowel anastomoses.

At the same time this opens up a field of investigation into the reasons why this should be so. Comparison between healing processes in small and in large bowel might yield clues to the mechanisms involved and, hopefully, produce clinically useful knowledge. First results from animal experiments indicate that at least under experimental conditions there are differences between the restorative processes in small and in large bowel (11).

In contrast to the low leakage rate under 'clean' conditions stands the appreciable rate under aggravating circumstances such as inflammation, vascular compromise and radiation damage. Here the rate for small bowel leakage is comparable to the rate in large bowel anastomosis, which may be put at at least 10,5% (1). It seems likely, therefore, that although the basic conditions in the small bowel are more favorable to sound healing of anastomoses, the aggravating factors are far more important. Because numerically inflammation is by far the more important of these, the detrimental influence of infection merits continued study (12,13).

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Chapter 7

TREATMENT OF SMALL BOWEL ANASTOMOTIC INSUFFICIENCY

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Submitted

7.1 Summary

In the period 1977-1981 we have treated 8 patients with anastomotic leakage and 9 patients with enterocutaneous fistula after small bowel resection. The results of oversewing and of resection with immediate reanastomosis were disappointing. Better results were obtained by dismantling of the anastomosis, establishment of a split-enterostomy and closure of a stoma in a second stage. Our overall mortality was 18% (3/17). The literature is reviewed.

7.2 Introduction

Dehiscence of an anastomosis in the small bowel is a major catastrophe. Kümmerle (1) described not long ago a series of six patients who all succumbed to this complication. Several different tactiques for treatment are proposed e.g. re-resection followed by re-anastomosis (2), drainage of an abscess in the expectation that a localised enterocutaneous fistula will develop (3) or the establishment of an enterostomy (4). Recently, good results have been described after a proximal diverting jejunostomy (5,6). For some years our preference has been the dismantling of the anastomosis and the construction of a split enterostomy (7). We present the results of this tactique in 14 patients.

7.3 Patients

From the beginning of 1977 to end 1981 243 small bowel

anastomoses were constructed in 143 patients over the age of 16 years. Patients were divided into four groups according to the presumed risks to an anastomosis associated with the underlying disorder as follows: I: vascular compromise (mesenteric thrombosis, incarcerated hernia); II: infection (diffuse peritonitis, localised abscesses); III: post-irradiation and IV: a groups designated as 'clean' (trauma, hemorrhage, obstruction).

In 14 patients (9.8% of total number) disturbed healing of anastomoses was seen. Three patients had anastomotic complications after two separate operations which brings the total number to 17 complications (7% of total anastomoses): 8 anastomotic dehiscences (3.3%) and 9 enterocutaneous fistulas (3.7%). The age of these patients ranged from 21 to 76 years. Dehiscences occurred in five ileocolostomies and in three ileoileostomie. Fistulas occurred in one ileo-colostomy and in eight ileoileostomies (Table 1). Underlying disorders and risk groups are given in Table 2.

7.4 Treatment

One case of anastomotic dehiscence was treated by reresection with a second ileoileostomy. Renewed leakage was treated by split-enterostomy. In the other seven cases the anastomosis was dismantled and a split-enterostomy constructed.

Table 1: *Incidence of leakage after small bowel resection*

	Ileo-ileostomy	Ileo-colostomy
Anastomotic leakage	3	5
Fistula	8	1

Table 2: *Disturbed healing of small bowel anastomosis in relation to risk factors*

	ANASTOMOTIC LEAKAGE		FISTULA	
Risk groups	Indication for operation	No. of patients	Indication for operation	No. of patients
Group I (n=26)	- incarcerated inguinal hernia	1		
	- mesenteric vascular occlusion	1		
Group II (n=55)	- M. Crohn	3	- M. Crohn	4
	- fistula	1	- diverticulitis	3
Group III (n=5)	- carcinoma of bladder	1	- carcinoma of bladder	1
Group IV (n=57)	- metastatic cancer	1	- trauma	1

Table 3: *14 patients with anastomotic dehiscence after small bowel resection. Treatment and results.*

Patient	Sex	Age	Risk group	Anastomosis	Complication	Treatment and complication	Stomal closure	Complication	Treatment
1	m	76	I	ii	leakage	ss	yes		
2	m	49	I	ic	leakage	ss - †			
3	m	59	II	ii	leakage	ii - leakage - ss	yes		
4	f	31	II	ic	leakage	ss	yes		
				ii	fistula	o			
5	f	44	II	ii	fistula	ii - fistula - ii			
			II	ic	fistula	ss	yes		
6	f	31	II	ic	leakage	ss	yes		
			II	ii	fistula	conservative			
7	m	46	II	ii	fistula	conservative			
8	m	21		ii	fistula	ss - †			
9	f	31	II	ic	leakage	ss	yes	fistula	ic
10	f	62	II	ii	fistula	ii			
11	m	26	II	ii	fistula	ss	yes		
12	m	68	III	ii	leakage	ss	yes	leakage	ss - †
13	m	72	III	ii	fistula	o - f - ii - leakage - ss	yes		
14	m	68	IV	ic	leakage	ss			

ii = ileo-ileostomy ss = split-stoma f = fistula

ic = ileo-colostomy o = oversewn

The fistulas were treated in various ways. Two patients without accompanying abdominal signs were treated successfully by conservative means. In two patients the fistulas were oversewn. Two patients were submitted to reresection and reanastomosis. Finally, in three cases with associated intra-abdominal abscesses, dismantling and split enterostomy were used (Table 3).

7.5 Results

Oversewing was successful in one out of two attempts. Reresection and reanastomosis was successful in one out of three attempts. In three patients resection and anastomosis was again tried later on in the course of the illness with one failure and thus the total success rate of this method was three out of six.

Two patients died after establishment of the enterostomy:
- a man of 49 years with mesenteric thrombosis of progressive intestinal necrosis;
- a boy of 21 years, a hemophiliac, of uncontrollable bleeding associated with necrotising fasciitis of the abdominal wall and an infected intra-abdominal hematoma.

In the final account 10 patients were eligible for restoration of the continuity of the gut after split-enterostomy (Table 3). In one patient we chose not to do this because of disseminated malignancy. In nine patients two anastomotic complications were seen: one fistula, cured after resection and anastomosis, one dehiscence for which again a split-enterostomy was constructed. This patient, suffering from radiation enteritis, died from massive pulmonary atelectasis after this last operation.

Overall mortality was thus 3 out of 17 cases (18%). anastomotic dehiscence (2/8; 25%) seems more dangerous than

fistulisation (1/9; 11%), but the small numbers preclude a firm conclusion.

7.6 Discussion

The aim of treatment of anastomotic leakage should be to eliminate the source of infection. Oversewing or reresection with reanastomosis are unreliable methods. Chances of renewed leakage have been reported as ranging between 50 and 70% (8,9) and our experience confirms this. Two other possible solutions are split-enterostomy after dismantling the anastomosis and proximal diverting enterostomy. We have chosen split-enterostomy. This demands a complete laparotomy with inspection of the intra-abdominal situation, localisation and drainage of all puspockets and the construction of a regular Brooke type end enterostomy. This way, the source of infection is completely eliminated.

We have no experience with diverting jejunostomy in this type of patients. It is a logical extension of the use of loop colostomy in comparable situations in colonic surgery. We have, however, found that a loop colostomy is not very reliable and use a Hartmann's or Mickulicz type of operation (the corollary of split-enterostomy) instead (10). Extended experience with split-enterostomy in all sorts of peritonitis has shown that one of the main difficulties is maintenance of fluid and electrolyte balance (11). Therefore, it may be advisable to reinstill the stomal effluent in the efferent limb and this may be hazardous in the case of diverting jejunostomy with pathology distal from it.

The overall mortality (including the mortality after closure of the stoma) was 18% which compares well with published figures (1,12,13), the more so if one takes into account that in two of the three fatalities sepsis as a (contributory) factor can be excluded.

In conclusion, we propose that in the treatment of anastomotic leakage in the small bowel there is a place for dismantling the anastomosis and establishing a split-enterostomy.

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Chapter 8

ENTEROSTOMY ON THE SMALL BOWEL AS AN ADJUNCT TO TREATMENT OF INTRA-ABDOMINAL SEPSIS

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Submitted

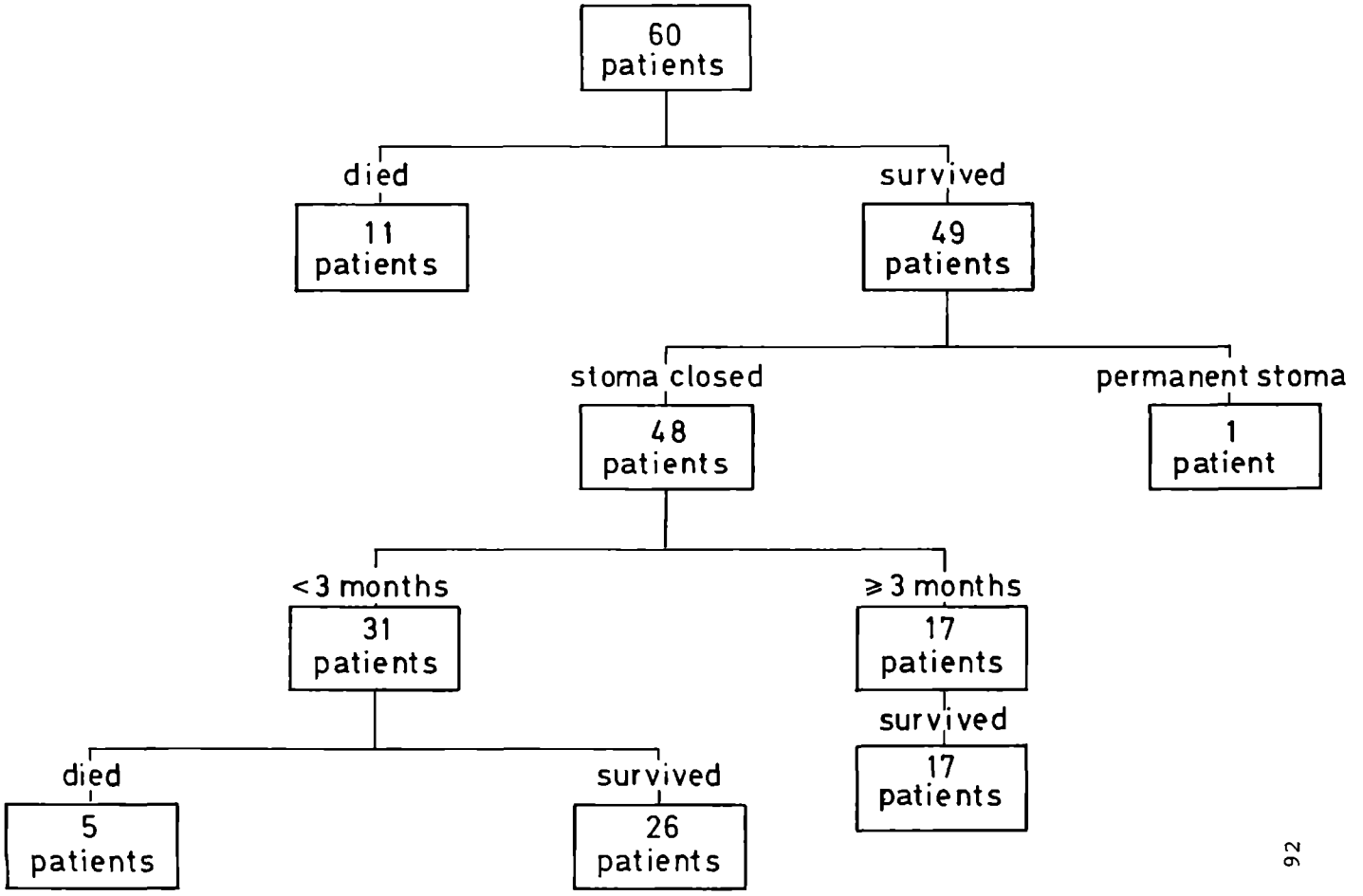
8.1 Summary

In 60 patients an enterostomy on the small bowel was constructed as part of the treatment of various intra-abdominal infectious and obstructive conditions: postoperative sepsis (n=31), primary peritonitis (n=16), obstruction and/or fistula (n=9) and miscellaneous (n=4). Eleven patients (18%) died in the immediate aftermath from continuing sepsis. In one patient closure of the stoma was not considered because of disseminated malignancy.

In 48 patients continuity of the gut was restored. In 22 patients (46%) complications occurred, 12 (25%) of which were intra-abdominal septic complications. The occurrence of intra-abdominal complications was found to be linked to premature (i.e. within three months) closure of the stoma. Reasons for premature closure were stomal difficulties and prerenal azotemia. Stomal closure was attended by a 10% mortality.

8.2 Introduction

Establishing a colostomy as prevention or treatment of anastomotic dehiscence is accepted practice in surgery of the colon. Several adverse circumstances, e.g. generalized peritonitis, intra-abdominal abscesses and impaired circulation, are held responsible for an increased leakage rate in colonic surgery. We have demonstrated that under


60 patients
died
11 patients
survived
49 patients
stoma closed
48 patients
permanent stoma
1 patient
< 3 months
31 patients
≥ 3 months
17 patients
survived
17 patients
died
5 patients
survived
26 patients

these conditions there is also an increase in the number of anastomotic failures in the small bowel (1). A logical extension of experience gained in colonic surgery would be to apply the principle of enterostomy to special problems in small bowel surgery. There have been occasional advocates of the use of enterostomy in Crohn's disease (2,3) and in necrotizing enteritis in children (4). Preliminary experience with its use in generalized peritonitis has been described by Mulholland et al (5) and by ourselves (6).

Apart from possible advantages, the method has obvious drawbacks: loss of fluid and electrolytes, loss of resorptive surface and the necessity of a further operation for re-establishment of the continuity of the gut. It is known that reconstruction of a colostomy is burdened with a morbidity ranging from 10 to 57% and a mortality in the order of 2% (7,8,9). As far as we know, there exist no data in the literature concerning morbidity and mortality concomitant to the use of enterostomy in small bowel surgery. Therefore, we present our experience with 60 patients.

8.3 Patients and methods

In the period 1977-1984 a split-enterostomy on the small bowel was established in 60 patients over the age of 16 years. There were 32 male patients and 28 female patients. Ages ranged from 19 to 76 years with a mean age of 45.7 (Figure 1). The mesentery of the bowel was divided as far as possible and necessary to bring out the afferent limb through a separate incision away from the main abdominal wound. A Brooke type ileostomy was always aimed at. The efferent limb was either brought out through the main wound or a separate wound, or oversewn and fixed to the parietal peritoneum to facilitate later retrieval. Patients were divided into four groups according to the presumed risk of the underlying disorder:
1. postoperative intra-abdominal sepsis;

Table 1: *Indications for establishing an enterostomy on the small bowel*

Group 1: Postoperative peritonitis		**n=31**
Anastomotic dehiscence		19
Underlying disorder		
sigmoid carcinoma	2	
mesenteric infarction	3	
bifurcation prothesis	1	
Crohn's disease	4	
incarcerated hernia	2	
irradiation enteritis	1	
multiple fistulas	1	
Bricker loop	3	
hemophilia	1	
myxoma peritonei	1	
Intra-abdominal abscesses		12
Underlying disorder		
gastric resection	2	
incarcerated hernia	1	
appendicectomy	5	
blunt abdominal trauma	1	
pancreatitis	1	
cholecystectomy	1	
retroperitoneal lymph-adenectomy	1	

Group 2: Primary peritonitis		**n=16**
Underlying disorder		
blunt abdominal trauma	2	
sigmoidperforation	1	
Crohn's disease	7	
incarcerated hernia	2	
ulcerative colitis	1	
perf. gallstone ileus	1	
mesenteric infarction	1	
perforated metastasis	1	
Group 3: Obstruction/Fistulas		**n= 9**
Underlying disorder		
postoperative fistula	2	
volvulus	1	
Crohn's disease	4	
laparoscopy	1	
rectovaginal fistula	1	
Group 4: Miscellaneous		**n= 4**
Underlying disorder		
GI hemorrhage in Crohn's disease	1	
irradiation enteritis	1	
necrotizing enteritis	1	
ingrown sigmoid carcinoma	1	

2. primary peritonitis (appendicitis and perforated gastric/ duodenal ulcer excluded);
3. obstruction or fistula;
4. miscellaneous (Table 1).

Eleven patients died after establishment of the enterostomy and in one patient no further surgery was performed because of the presence of disseminated malignancy. Continuity of the gut was restored in 48 patients. Bowel preparation was not practiced and antibiotic cover was not routinely given for these small bowel operations. Anastomoses were of the two-layer kind with an outer knotted layer and an inner running suture of Vicryl^{R} 3-0. Complications were tabulated and correlations were sought with the following factors: albumin level, renal function (urea, creatinin), liver function (bilirubin, alkaline phosphatase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase) and time elapsed between construction of the stoma and reestablishment of continuity of the gut.

8.4 Results

Eleven patients died after establishment of the enterostomy from irreversible intra-abdominal sepsis and multiple organ failure. Mean age of these patients was 56 years (range 21-73 years). Nine patients belonged to group 1 and two to group 2.

Of 48 patients 21 could leave the hospital for further recovery at home. Two of these had to be readmitted earlier than foreseen because of dehydration, while 19 could be re-admitted for closure of the stoma on an elective basis. Twenty-nine patients needed in-hospital treatment i.a. because of the necessity of fluid and electrolyte replacement. Loss from the stoma measured more than 1 l in 6 patients, more than 1.5 l in 7 patients, more than 2 l in 8 patients

Table 2: *Number and localisation of anastomoses*

Jejuno-jejunostomy	11
Jejuno-ileostomy	1
Jejuno-colostomy	1
Ileo-ileostomy	31
Ileo-colostomy	14
Colo-colostomy	4

(main wound closed 20, open 28; stomal wound closed 6, open 42)

Table 3: *Complications of stomal closure in 22 patients*

Wound infection	6
Pneumonia	1
Pulmonary embolism	1
Dehiscence abdominal wound	2
Intra-abdominal abscess	2
Bladder fistula	1
Enterocutaneous fistula	2
Dehiscence anastomosis	7

and more than 3 l in 2 patients. For the remaining 6 patients the exact amount could not be calculated from the notes. Nineteen patients received parenteral nutrition as well as fluid replacement.

In these 48 patients 62 bowel anastomoses were constructed (Table 2). Complications occurred in 22 patients (46%; Table 3). Wound complications were seen exclusively in those patients where the skin and subcutaneous tissues of the wound had been closed. Intra-abdominal complications were seen in 12 patients (25%). It is to be noted that in two patients an intra-abdominal abscess occurred which was caused by a bowel

Table 4: *Time interval between construction and closure of stoma related to the occurrence of complications.*

Time interval	Number of patients without complications	Number of patients with complications
2-4 weeks	3	5 (4)*
4-8 weeks	6	7 (3)
8-12 weeks	3	7 (5)
>3 months	6	2
>4 months	4	
>6 months	3	
>9 months	1	1

* number of patients with intra-abdominal complications

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perforation at some distance from the intact anastomosis. Intra-abdominal complications occurred exclusively in patients where the stoma was closed within three months (Table 4). The time elapsed between stomal construction and closure was significantly less ($p=0.005$, Wilcoxon test for two random tests) in patients with intra-abdominal complications than in patients where no complications occurred. More generally, this time interval was also significantly ($p=0.017$) smaller in patients with complications than in patients without complications. Disturbances in renal function and in liver function showed no statistically significant relation with the emergence of complications. A serum albumin level of less than 35 g/l was present in 47% of patients with intra-abdominal complications against 9% of patients without these complications ($p=0.01$, Fishers two-sided exact test for 2 x 2 table).

The further course of the patients with intra-abdominal complications is given in Table 5. The mean age of the five

Table 5: *Intra-abdominal complications after closure of split-stomata. Treatment and results.*

Patient	Age	Sex	Treatment and complications	Results
Fistula and abscess				
1	21	m	D+O	AW
2	49	m	D+R/A	AW
Enterocutaneous fistula				
3	44	f	Cons+R/A	AW
4	31	f	Cons+R/A	AW
5	64	f	Cons	†
Anastomotic dehiscence				
6	21	m	O L SS A L SS A	AW
7	25	f	SS A	AW
8	50	f	R/A	AW
9	68	m	SS	†
10	64	f	O	†
11	68	m	SS	†
12	60	f	O L SS R/A L R/A L R/A L SS	†

D = drainage; O = oversewn; R = resection; A = anastomosis; Cons = conservative; SS = split-stoma; L = leakage; AW = alive and well; † = died

patients who died was 63.5 years which was significantly (p=0.008, Wilcoxon) higher than the mean age of the patient who survived. The mortality related to closure of the enterostomy was 10.4% and occurred exclusively in patients belonging to group 1. Total mortality in group 1 was 14/31 (45%); in group 2 2/16 (12.5%) and in groups 3 and 4 nil. Due to the relatively small numbers this obvious trend could not be shown to be statistically significant.

8.5 Discussion

Establishment of an ileostomy or a jejunostomy as part of the treatment for the conditions from which this series of patients suffered is sound in theory. Suturing the bowel under septic conditions carries a high risk, as again demonstrated by the fact that in this series oversewing of small leaks was followed by renewed leakage in more than 50% of iatrogenic lesions and in 100% of anastomotic leaks. In practice, however, considerable difficulties are encountered. In at least 17 patients (35%) daily fluid loss was over 1.5 l/24 hrs which necessitated intensive supervision of fluid balance under hospital conditions. Even then, correction was not always achieved, as will be discussed later.

Closure of the enterostomy was accompanied by a complication rate of 46%. The complications can be divided into intra-abdominal complications and 'other' complications. Among the other complications wound infection was the most common and was seen when the wound was closed completely. Wound infection did not occur when the skin was left open, whilst hospital stay was not prolonged by this practice. The occurrence of wound infection in these patients may thus be largely preventable. Intra-abdominal complications were found to be significantly correlated to time interval between construction and closure of the stoma and to serum albumin level. Both

are obviously related to the clinical course and condition of the patients. Those patients (19) who could be sent home and readmitted electively constitute a favourable selection: no continuing infection, easily correctable fluid loss, normal oral intake and thus a normal albumin level. No intra-abdominal complications after closure of the stoma were seen in this group. The two major reasons for premature (i.e. within three months) closure of the enterostomy in the in-hospital group were stomal difficulties and prerenal azotemia. The stoma has often to be established under sub-optimal conditions: emergency operation; mesentery infiltrated, thickened and shortened by intraperitoneal infection; loops of bowel glued together by dense adhesions; site of stoma in the bowel dictated by the site of the leak; choice of position of stoma in the abdominal wall limited by scars from previous incisions and drains. All this may result in technically deficient stomas posing problems in care which even an experienced stomal therapist can not overcome. Our practice has been to restore continuity in these cases, but in the light of the experience presented here we think that an attempt at refashioning of the stoma might be wiser. In 14 patients the reason for premature closure of the stoma was prerenal azotemia, notwithstanding careful attention to replacement. The same problem has been encountered in neonatal necrotizing enterocolitis (10). In these children this 'salt and water losing state' has been attributed to loss of absorptive function of the remaining intestine (11). This could conceivably be an important factor also in adult patients. The obvious remedy would be reinstilling the stomal effluent into the efferent limb. So far, we have done this only occasionally because it is rather cumbersome, but having realized the frequency of the problem and the increased risk attached to premature closure of the stoma, we propose to use this technique more often. The mortality of closure (10.4%) is equal to the 10% reported by Cogbill and Millikan (10) in children. Thus, establishing an enterostomy in the

type of patients discussed in this paper is fraught with difficulties.

This retrospective study has allowed to detect three ways, in which the frequency of complications may be diminished: attention to construction of the stoma and where possible refashioning instead of premature closure; attention to fluid balance, where possible aided by reinstilling the effluent; realisation that restoration of continuity has the best prospects if the patient is in a stable phase and that it probably takes at least three months to reach this phase. The question remains whether applying the principle of enterostomy under adverse circumstances alters in any way the course of the disease. A mortality of 45% in the patients in group 1 is staggering, although it has to be realised that this is a highly selected group and that not long ago a mortality of 100% has been reported (12) for this type of patients. We have, frankly, found this question impossible to answer. Especially in postoperative peritonitis there are so many variables to consider that it is impossible to single out one of them and attribute an eventually found increased survival to this one variable alone. For the same reason clinical trials are hardly feasible, apart from ethical considerations. Remains the fact, then, that in many of the situations encountered in the patients concerned, there is hardly any other way out.

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Lekkage van darmnaden vormt een ernstig klinisch probleem en gaat gepaard met hoge morbiditeit en mortaliteit. In tegenstelling tot de uitgebreide documentatie in de literatuur omtrent complicaties bij colonnaden ontbreken gegevens over lekkage van dunne darmnaden vrijwel volledig. Oorzaak hiervan zou kunnen zijn dat naadlekkages in de dunne darm niet of nauwelijks voorkomen, dan wel klinisch van weinig betekenis zijn.

Bij onderzoek naar het collageenmetabolisme rond experimentele colonnaden is gebleken dat de collageenconcentratie na het aanleggen van de naad eerst sterk afneemt en zich vervolgens herstelt. Dit tijdelijke verlies aan collageen zou uiteindelijk ten grondslag liggen aan een optredende naadlekkage. Over de eventuele veranderingen in collageen rond experimentele naden van de dunne darm is niets bekend.

Dit proefschrift beschrijft enerzijds een onderzoek waarin de wondgenezing van colon- en ileumnaden wordt vergeleken in het konijn (hoofdstukken 1-5) en anderzijds een retrospectief onderzoek naar incidentie, preventie en behandeling van lekkages van dunne darmnaden in onze kliniek (hoofdstukken 6, 7 en 8).

In eerste instantie zijn de veranderingen in collageenconcentratie, gemeten als hydroxyproline en uitgedrukt per mg droog gewicht, en de veranderingen in bursting pressure, als maat voor de sterkte van de darm, vergeleken vanaf 3 uur na operatie (hoofdstukken 1 en 2). Zowel in het colon als in het ileum treedt er na het aanleggen van een anastomose een tijdelijk verlies op in sterkte van de darmwand ter plaatse van de naad: de bursting pressure is significant verlaagd ten opzichte van die in intacte darm. Dit verlies van sterkte van de darmwand gaat gepaard met een forse daling in het

collageen gehalte van de darm. Echter, er treden significante kwantitatieve verschillen op tussen ileum en colonnaden. In het colon is het hydroxyproline gehalte in de naad reeds 3 uur postoperatief significant gedaald: na 48 uur bereikt dit zijn minimum waarbij de concentratie met 38% is afgenomen ten opzichte van de intacte darm. Hierna treedt een geleidelijk herstel op, zodanig dat na 7 dagen de uitgangsituatie weer wordt bereikt. In het ileum daarentegen treedt pas na 12 uur een significante daling op van het hydroxyproline gehalte van de naad. Na 24 uur bedraagt het maximale verlies 27%. Hierop volgt een spoedig herstel. Na 7 dagen wordt een zelfs veel grotere concentratie van hydroxyproline gevonden dan in de oorspronkelijke darm aanwezig was. Als nu het optredende verlies aan hydroxyproline concentratie in colon- en ileumnaden met elkaar wordt vergeleken met behulp van tweezijdige variantie analyse, dan blijkt het verlies aan hydroxyproline in colonnaden systematisch groter te zijn dan in ileumnaden. In tegenstelling met het colon, waar ook in darmsegmenten proximaal en distaal van de naad veranderingen optreden in het hydroxyproline gehalte, blijft dit in het ileum uitsluitend beperkt tot de naad zelf. De concentratie van totaal eiwit rond de naad verandert niet significant, noch in ileum noch in colon.

Deze resultaten duiden erop dat het collageengehalte in ileum- en colonnaden op een kwalitatief gelijksoortige wijze verandert. Kwantitatief zijn er echter belangrijke verschillen: in het ileum is het collageenverlies minder groot, het blijft beperkt tot het gebied vlak rond de naad en het herstelt zich sneller dan in het colon. Dergelijke verschillen kunnen mogelijk verklaren waarom het genezingsproces van een dunne darmnaad vaker ongestoord verloopt dan dat van een naad in de dikke darm.

Uit klinisch onderzoek is gebleken dat het leggen van darmnaden in geinfecteerd milieu leidt tot een verhoogde kans

op naadlekkage. Om het effect van infectie op wondgenezing in dikke en dunne darm te onderzoeken is een model ontwikkeld waarbij in het konijn een experimentele peritonitis wordt opgewekt met behulp van implantatie van gelatine capsules met humane faeces (hoofdstuk 3). Dit model onderscheidt zich van andere modellen voor experimentele peritonitis, doordat het de mogelijkheid biedt, bij een juiste keuze van de hoeveelheid infectieus materiaal, een voldoende lange overlevingsduur van aantoonbaar geïnfecteerde dieren te bereiken om het proces van de naadgenezing gedurende tenminste de eerste, cruciale, week te bestuderen.

De negatieve invloed van een intra-abdominale infectie op het genezingsproces van een darmnaad wordt niet alleen gedemonstreerd door een aanzienlijk verlaagde (ten opzichte van niet-geïnfecteerde darm) barsting pressure van colon- en ileumnaden, maar ook door het feit dat in een groot aantal colon- en ileumnaden de lekkage na 7 dagen nog optreedt in de naad zelf, terwijl op dit tijdstip in de niet-geïnfecteerde groep alle naden in zowel ileum als colon sterker zijn dan hun omgeving (hoofdstuk 4). Ook het verlies aan collageen in colon- en ileumnaden is in de geïnfecteerde groep groter dan in de niet-geïnfecteerde groep. Door de aanwezigheid van de infectie zal de lengte van het stuk darmwand waarin collageen-afbraak optreedt worden vergroot.

Het genezingsproces van de experimentele darmnaden is tenslotte histologisch onderzocht (hoofdstuk 5). Dit proces wordt in beide delen van de darm gekarakteriseerd door een vast patroon van elkaar opvolgende celinfiltraties. Twaalf uur na de operatie wordt het histologische beeld van de wand beheerst door een massale infiltratie van granulocyten. Aangezien er op dat moment in beide delen van de darm reeds een significant verlies aan collageen is opgetreden en het bekend is dat granulocyten het enzym collagenase bevatten, behoort het tot de mogelijkheden dat de granulocyt de afbraak van collageen

bewerkstelligt. Na 7 dagen worden significante verschillen gevonden tussen colon- en ileumnaden wat betreft het herstel van de mucosa, het opruimen van de necrose en de aanwezigheid van granulocyten. Het na 7 dagen nog aanwezig zijn van collagenase bevattende granulocyten in colonnaden zou een trager herstel in collageengehalte kunnen verklaren.

Om de incidentie van naadlekkage in de dunne darm in onze kliniek te onderzoeken is een retrospectieve studie verricht (hoofdstuk 6). In de periode 1977-1982 werden bij 143 patienten 243 dunne darmanastomoses aangelegd. De volgende risicogroepen werden onderscheiden: intra-abdominale ontsteking, vasculaire stoornissen en bestraling. In afwezigheid van bovengenoemde risicofactoren, de zogenaamde 'schone' groep, trad slechts in 0,8% van de gevallen een naadlekkage op. Dit is aanzienlijk lager dan gewoonlijk in de literatuur wordt aangegeven voor vergelijkbare patienten met colonnaden (3-5%). Bij aanwezigheid van een intra-abdominale ontsteking werd een significant hoger percentage naadlekkages van 14,8% gevonden, vergelijkbaar met de opgegeven frequentie (10-20%) voor colonnaden onder deze omstandigheden. De conclusie lijkt gerechtvaardigd dat onder normale omstandigheden een naadlekkage in de dunne darm een zelden voorkomende complicatie is. Echter, onder minder gunstige omstandigheden is de incidentie gelijk aan die optredend bij colonnaden.

Voor de behandeling van naadlekkage (hoofdstuk 7), maar ook van enterocutane fistels met tekenen van een locaal infect, geven wij, gezien de grote kans op een recidief lekkage na simpel overhechten of het aanleggen van een nieuwe naad, de voorkeur aan het ontmantelen van de naad en aanleggen van split-stomata. De overall mortaliteit bedraagt 18%. Op de voor- en nadelen van deze behandeling wordt verder ingegaan. Door het aanleggen van een split-stoma wordt het risico van recidief naadlekkage vermeden. Na enige tijd, indien de omstandigheden gunstiger zijn, kan de continuiteit van de darm

weer worden hersteld. Het aantal naadlekkages bij deze laatste ingreep is 15%.

In hoofdstuk 8 tenslotte worden de resultaten weergegeven van het opheffen van split-stomata. Dat deze laatste fase in de behandeling niet van gevaren ontbloot is wanneer de omstandigheden niet optimaal zijn, blijkt uit een optredende mortaliteit van 10,4%. In 25% van de gevallen traden septische intra-abdominale complicaties op, uitsluitend bij patienten waarbij het stoma binnen de 3 maanden na de laatste abdominale operatie werd opgeheven. De invloed van verschillende risico-factoren werden nagegaan. De oorzaken van het gedwongen vervroegd opheffen van de split-stomata (binnen 3 maanden) blijken vooral te bestaan uit technische problemen met betrekking tot het stoma en problemen met betrekking tot de vocht- en electrolytenbalans. Nauwlettende aandacht voor deze twee aspecten kan mogelijkerwijs deze resulaten verbeteren.

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- Centraal Dierenlaboratorium
- Research Laboratorium Heelkunde
- Pathologische Anatomie
- Medische Bibliotheek
- Medische Fotografie en Illustratie
- Mathematisch Statistische Adviesafdeling.

CURRICULUM VITAE

De auteur werd op 18 maart 1951 geboren te Nijmegen. In 1969 behaalde hij het HBS-B diploma aan het Canisius College te Nijmegen. In die stad studeerde hij vervolgens geneeskunde aan de Katholieke Universiteit. Tijdens zijn studie was hij werkzaam als assistent op de afdeling Anatomie en in het kader van de stage sociale geneeskunde werkte hij in 1977 gedurende 5 maanden in het Bukumbi Ziekenhuis in Tanzania. In 1977 voltooide hij zijn studie met het behalen van het artsexamen. Als algemeen arts-assistent werkte hij nadien gedurende 7 maanden in het Bethesda Ziekenhuis in Tiel. Van 1 mei 1978 tot 1 mei 1984 specialiseerde hij zich in de algemene heelkunde (opleider: Prof.Dr. H.H.M. de Boer). Sinds 1981 is hij lid van de medische commissie van de Koninklijke Nederlandse Motorrijders Vereniging (KNMV). Tijdens het 8th World Congress van het Collegium Internationale Chirurgiae Digestivae was hij co-chairman bij de free paper session 'Anastomosis'. Hij publiceerde in binnen- en buitenlandse tijdschriften over verschillende chirurgische onderwerpen, zoals diverticulitis van het colon, cystadenoma van de epididymis, achtergebleven corpora aliena in de buik, voetfracturen, instabiele bekkenfracturen en wondgenezing in de darm.

STELLINGEN

I

Geringere afname en sneller herstel van de collageen-concentratie in een naad van de dunne darm in vergelijking tot de dikke darm verklaart het minder frequent voorkomen van een naadlekkage (dit proefschrift).

II

Indien geen risicofactoren aanwezig zijn lekt een dunne darmnaad zelden (dit proefschrift).

III

Gezien de grote kans op hernieuwde lekkage dienen bij patienten met intra-abdominale sepsis iatrogene laesies, fistels en lekkende naden niet overhecht te worden (dit proefschrift).

IV

De continuiteit van de darm bij patienten met een dubbel stoma behoort bij voorkeur pas na drie maanden hersteld te worden.

V

Op grond van gegevens verkregen uit experimenteel onderzoek heeft een eenrijige geknoopte inverterende darmnaad de voorkeur.

VI

Bij een patient met een tumor in de buik, bij wie in het verleden een laparotomie is verricht, dient een achtergebleven corpus alienum in de differentiaal diagnose te worden opgenomen.

VII

Het bestaan van een fistel bij een diverticulitis van het colon duidt op een onvoldoende drainage van het ontstekingsproces.

VIII

Het bilaterale papillaire cystadenoma van de epididymis, waarbij in de familie een cerebellair hemangioblastoma voorkomt, moet beschouwd worden als de epididymale manifestatie van de ziekte van Lindau. Intensieve controle is noodzakelijk.

IX

Een anatomische repositie en goede fixatie van fracturen en luxaties in het Lisfranc gewricht geeft de beste kans op een goed functioneel eindresultaat.

X

Een instabiele bekkenfractuur, een letsel dat meestal bij multitrauma patienten wordt aangetroffen, dient, evenals de overige fracturen, in de vroege fase van de behandeling gestabiliseerd te worden.

XI

Met de introductie van de flexibele sigmoideoscoop is de starre scoop obsoleet geworden.

XII

Bij jonge patienten met een goed gedifferentieerd schildklier-carcinoom kan volstaan worden met een hemithyreoidectomie.

XIII

Het aantal en de ernst van letsels opgelopen tijdens wegraces met motoren blijkt niet te correleren met de snelheid die op de verschillende circuits wordt bereikt.

XIV

De enige vorm waarin een naadinsufficiëntie toch nog een gunstig resultaat kan hebben is 'je uit de naad te werken'.